Interaction Between Polymorphisms of IFN-γ and MICA Correlated with Hepatocellular Carcinoma

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Background: We explored the relationship of interferon-γ (IFN-γ) and MHC class-I chain related gene A (MICA) genes polymorphisms with hepatocellular carcinoma (HCC) risk, and tried to determine whether the interaction existed between these two genes polymorphisms on the basis of HCC.

Material/Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the genotypes of the 3 single-nucleotide polymorphisms (SNPs) and to analyze the correlation of each SNP with HCC susceptibility in 120 HCC patients and 124 healthy people. The association strength between the 3 SNPs and HCC is represented with odds ratio (OR) and 95% confidence interval (95% CI). Hardy-Weinberg equilibrium (HWE) was tested by $\chi^2$ test in the control group.

Results: GG genotype of IFN-γ rs2069727 polymorphism had apparently different distributions in case and control groups ($P<0.05$), and might confer increased risk of HCC (OR=3.40, 95%CI=1.23–9.38). Analysis of MICA rs2596542 polymorphism also yielded the same result (OR=2.90, 95%CI=1.10–7.67), as did their risk alleles. Specifically, the interaction between rs2596542 and rs2069705 polymorphisms increased the HCC risk by 1.41 times and between rs2596542 and rs2069727 polymorphisms the increased risk of HCC by 5.56 times.

Conclusions: IFN-γ rs2069727 and MICA rs2596542 polymorphisms may be related to the incidence of HCC. Interaction exists between the polymorphisms of IFN-γ and MICA, which may increase risk of HCC.

MeSH Keywords: Carcinoma, Hepatocellular • Epistasis, Genetic • Interferons • Polymorphism, Genetic

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**Background**

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor worldwide and it is widely spread throughout the world [1]. Every year, there are about 500,000–1,000,000 people newly diagnosed with HCC and about 620,000 people die from HCC-related diseases [2,3]. According to the preliminary data recently collected by the WHO, China is one of the countries with high incidence of HCC, and as much as 55% of new HCC cases worldwide come from China in [4–6]. Early symptoms of HCC are easily ignored and only 3–5% of the confirmed HCC cases survive longer than 5 years. The pathogenesis of HCC is still unclear, but studies have indicated that inflammation plays an important role in its development [7]. Cytokines are major inflammation response mediators. It is a gene single-nucleotide polymorphism (SNPs) that can cause individual differences in cytokine levels and immune functions through affecting the transcription, translation, and expression of cytokines and the heterogeneity in cytokine genes may become markers for HCC susceptibility [8].

The interferon (IFN) is a kind of cytokine that was discovered and used relatively early. It is an inducible protein with a wide range of antiviral, antineoplastic, and immune regulation functions, and the exertion of numerous bioactivities depends on regulation of cell the genome. Many recent studies have found that polymorphisms in the noncoding region of IFN gene are associated with autoimmune response, chronic inflammation, and as tumor formation [9]. Through changing transcription factor binding sites, polymorphisms of IFN-γ gene can affect the expressions of related genes and lead to individual differences in cytokine levels and immune function [10].

**Material and Methods**

**Materials of the study**

As cases we selected 120 HCC patients (72 males and 48 females) with an average age of 56.78±13.3 diagnosed through histopathological or clinical examinations in Shandong Provincial Hospital affiliated to Shandong University from May of 2012 to September of 2014; they had no the history of other tumors. The 124 healthy controls with a mean age of 60.25±11.2 were 68 men and 56 women selected from healthy people at the Physical Examination Center in the same hospital the cases came from. They were with no primary liver cancer, hepatitis, liver cirrhosis, or hepatic distomiasis. Blood relationship did not exist among any subjects. The subjects volunteered to participate in the epidemiological questionnaire investigations on HCC and blood collection process. Informed consents were provided by every subject and the study strictly adhered to the ethics guidelines of the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University.

**Sample collection**

We collected 2 ml of elbow vein peripheral blood with ethylene diamine tetraacetic acid K2 (EDTA-K2) anticoagulation. Improved Nal method was applied to extract genomic DNA from leukocytes of the peripheral blood, and then the DNA samples were stored at –20°C.

**Primers design and PCR reaction conditions**

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to perform the genotyping of IFN-γ rs2069705, rs2069727, and MICA rs2596542 polymorphisms. Forward and reverse primer sequences were: rs2069705: 5’-TAGCAGCTTTATGAGGATTAC-3’ and 5’-AGGTAATCCTCATAAAGTGC-3’; rs2069727: 5’-AGGTTCTGCTATGGAATGTA-3’ and 5’-AAACTACATTCCATAGCAGA-3’; and rs2596542: 5’-TCGTCTCCAAAAGACCGTAC-3’ and 5’-CCGTCGTCGGGACCAACTGTC-3’. There were 1.0 µL DNA template, 12.5 µL 2×PCR Mix, each of 1.0 µL forward and reverse primers, and 9.5 µL deionized water in the 25 µL PCR reaction system. The PCR reaction conditions included: predenaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s; and finally extension at 72°C for 7 min. PCR amplification products were checked with 2% agarose gel electrophoresis (AGE).

The PCR amplification products of the 3 SNPs were digested with Pvu II, Hinf I, Alu I restriction enzymes and separated by 3% AGE.

**Statistical methods**

SPSS 18.0 software was used for statistical analysis. The χ² test was performed to compare the genotypes distributions of these 3 SNPs between the cases and controls. Hardy-Weinberg equilibrium (HWE) was tested. Odds ratios (OR) with 95% confidence intervals (95% CI) were used to represent the
relationship between the 3 SNPs and HCC susceptibility. The differences were regraded to have statistical significance only when the P value was smaller than 0.05. The interaction analysis was also completed by the $\chi^2$ test.

Results

HWE test

Through the $\chi^2$ test, the genotype distributions of IFN-\(\gamma\) rs2069705, rs2069727, and MICA rs2596542 polymorphisms in 120 cases and 124 controls were proved to conform to HWE, and this suggested that the selected subjects had representativeness.

Correlation between SNPs of IFN-\(\gamma\) and MICA and HCC

The linkage between IFN-\(\gamma\) and MICA polymorphisms and HCC is detailed in Table 1. Specifically, GG genotype of IFN-\(\gamma\) rs2069727 was more frequently distributed in cases than in controls, and differences between these 2 groups were statistically significant ($P<0.05$). Compared with AA genotype, GG increase by 2.4 times the risk of developing HCC (OR=3.40, 95% CI=1.23–9.38). The distributions of G allele in the 2 groups were also significantly different ($P<0.05$), indicating that G allele was a factor conferring susceptibility to HCC. However, no apparent relationship of rs2069705 polymorphism in IFN-\(\gamma\) gene with HCC was detected ($P>0.05$).

Statistical significance was discovered in AA genotype distribution of MICA rs2596542 polymorphism between case and control groups ($P<0.05$), and AA genotype was positively correlated with the onset risk of HCC (OR=2.90, 95% CI=1.10–7.67). The A allele of rs2596542 conferred higher risk for HCC than the G allele, and might also be a susceptibility factor for HCC (OR=1.57, 95% CI=1.07–2.31).

Association analysis of interaction between the 3 SNPs and HCC

The interaction between the 3 SNPs and the onset risk of HCC is shown in Table 2. The interaction between the mutant genotype of rs2596542 and rs2069705 polymorphisms raised the risk of HCC onset by 1.41 times (OR=2.41, 95% CI=1.09–5.31). In rs2596542 and rs2069727 polymorphisms, these 2 mutant

| Genotype/Allele | Case n=120 (%) | Control n=124 (%) | $\chi^2$ | $P$ | OR (95% CI) |
|-----------------|---------------|-------------------|---------|----|-------------|
| IFN-\(\gamma\) rs2069705 (C/T) | | | | | |
| CC | 59 (49.2) | 69 (55.6) | – | – | 1.00 |
| CT | 51 (42.5) | 49 (39.5) | 0.541 | 0.505 | 1.22 (0.72–2.06) |
| TT | 10 (8.3) | 6 (4.9) | 1.534 | 0.290 | 1.95 (0.67–5.68) |
| C | 169 (70.4) | 187 (75.4) | – | – | 1.00 |
| T | 71 (29.6) | 61 (24.6) | 1.537 | 0.223 | 1.28 (0.86–1.92) |
| rs2069727(A/G) | | | | | |
| AA | 50 (41.7) | 68 (54.8) | – | – | 1.00 |
| AG | 55 (45.8) | 50 (40.4) | 2.234 | 0.142 | 1.50 (0.88–2.54) |
| GG | 15 (12.5) | 6 (4.8) | 6.05 | 0.018 | 3.40 (1.23–9.38) |
| A | 155 (64.6) | 186 (75) | – | – | 1.00 |
| G | 85 (35.4) | 62 (25) | 6.29 | 0.014 | 1.65 (1.11–2.43) |
| MICA rs2596542 (A/G) | | | | | |
| GG | 48 (40) | 65 (52.4) | – | – | 1.00 |
| GA | 57 (47.5) | 52 (41.9) | 2.144 | 0.179 | 1.48 (0.87–2.52) |
| AA | 15 (12.5) | 7 (5.7) | 4.89 | 0.035 | 2.90 (1.10–7.67) |
| G | 153 (63.8) | 182 (73.4) | – | – | 1.00 |
| A | 87 (36.2) | 66 | 5.263 | 0.025 | 1.57 (1.07–2.31) |

Table 1. Comparison of genotype and allele distributions of IFN-\(\gamma\) rs2069705, rs2069727, and MICA rs2596542 based on HCC.
Table 2. Association of interaction between polymorphisms of IFN-γ and MICA genes with HCC.

| SNP   | SNP   | Case | Control | OR (95% CI) | P value |
|-------|-------|------|---------|-------------|---------|
| rs2596542 | rs2069705 | 27   | 26      | 1.00        | −       |
| +     | +     | 21   | 39      | 0.52 (0.24–1.10) | 0.127   |
| −     | +     | 32   | 43      | 0.72 (0.35–1.45) | 0.374   |
| −     | −     | 40   | 16      | 2.41 (1.09–5.31) | 0.032   |
| rs2596542 | rs2069727 | 8    | 21      | 1.00        | −       |
| +     | +     | 40   | 44      | 2.39 (0.95–5.99) | 0.081   |
| −     | +     | 42   | 47      | 2.35 (0.94–5.85) | 0.084   |
| −     | −     | 30   | 12      | 6.56 (2.29–18.83) | 0.001   |

“+” represents wild genotype, and “−” represents mutant genotype.

Discussion

China is a country with a high HCC incidence that accounts for about 55% of the global incidence. Every year, the morbidity and mortality of HCC in China are about 340,000 and 300,000, respectively, with an upward trend nationally [14]. The initiation and development of HCC are multifactorial and sophisticated biological processes involving environmental and genetic factors. Recently, association studies on gene polymorphisms and HCC susceptibility have attracted increasing attention. Genetic backgrounds can to a great extent determine the risk of individuals developing HCC [15,16].

Some studies have reported that the expression levels of soluble MICA are significantly increased in the serum of patients with HCC and chronic liver diseases [17,18]. Because the MICA gene is expressed in most epithelial cancer cells, its relationship with tumors has become a research focus for the past few years. Studies of the MICA gene have focussed on the relevance of alleles with tumors and the expression of MICA molecules on the surface of tumor cells. Studies carried out in European populations have discovered that associations exist between MICA gene and biliary cirrhosis [19,20]. Hoshida et al. found that MICA is related to hepatitis C-related HCC in Japanese [21]. It has been indicated by Chen et al. that the expression of MICA mRNA was reduced in HCC patients [22]. Therefore, we performed this study to explore the polymorphisms of MICA in Chinese Han HCC patients.

IFN is a multifunctional cytokine which has effects on the treatment of tumors and many other diseases [23]. Many recent studies in China and abroad have explored the association between IFN-γ polymorphisms and some tumors, autoimmune diseases, and infectious diseases (e.g., tuberculosis, hepatitis B, and leishmaniasis) of human [24-26]. Nevertheless, results on the relationship between IFN-γ polymorphisms and the occurrence of HCC are inconsistent.

The present study investigated rs2069705 and rs2069727 polymorphisms in IFN-γ and rs2596542 polymorphism in MICA and found that the distributions of the homozygous mutant genotype of IFN-γ rs2069727 polymorphism in case and control groups were significantly different. However, IFN-γ rs2069705 polymorphism was irrelevant to the onset of HCC. As for MICA rs2596542 polymorphism, its AA genotype could increase the onset risk of HCC and it was correlated with the incidence of the disease. Further gene-gene interaction analysis showed that the interaction between the mutant genotypes of IFN-γ rs2069705 and MICA rs2596542 polymorphisms could influence the onset of HCC. Interaction was shown between polymorphisms of rs2069727 and rs2596542, and HCC risk could be enhanced by the interaction.

Our study results show that IFN-γ and MICA polymorphisms affect the incidence of HCC, and the gene-gene interaction between them plays an important role in the occurrence and development of such disease. Therefore, we should pay more attention to the influences of IFN-γ and MICA genes on HCC.

Conclusions

Although we obtained statistically significant results, they are insufficient to understand the pathogenesis of HCC.
study had some limitations. Firstly, the sample size was small. Secondly, HCC is affected by multiple genetic and environmental factors, but environmental factors were not included in this study. Thirdly, the results were unadjusted. Finally, only 1 ethnicity was included. Therefore, a well-designed cohort study is needed, which should include a large sample size, more ethnicity gene–gene and gene-environment interactions, and adjustment for confounding factors. To confirm our results, another study focused on the MICA rs2596542 polymorphism and IFN-γ protein expression level is necessary. Only in this way can we provide more comprehensive guidance and a control strategy for the prevention of HCC.

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