MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 549-553
DOI: 10.12659/MSM.895101

Received: 2015.06.22
Accepted: 2015.08.25
Published: 2016.02.19

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

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AD 1 **Hongguang Li**
BC 1 **Fangfeng Liu**
DE 1 **Huaqiang Zhu**
DE 1 **Xu Zhou**
CF 1 **Jun Lu**
EF 1 **Hong Chang**
CFG 2 **Jinhua Hu**

1 Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, P.R. China
2 Department of Gastroenterology Surgery, Shandong Provincial Hospital affiliated to Shandong University, Jinan, Shandong, P.R. China

Corresponding Author: Jinhua Hu, e-mail: liyubanlk@sina.com
Source of support: Departmental sources

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 χ^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

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895101

1693 2 — 26

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

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].

MHC class-I chain-related gene A (MICA) gene is a member of the major histocompatibility complex (MHC) gene family. It is a functional gene with low expression levels in normal human histocytes [9]. Reports have shown that the *MICA* gene is expressed in the primary epithelial tumors cells, including HCC, lung cancer, breast cancer, and prostatic cancer [11–13]. Although some studies have confirmed the high expressions of *MICA* and *IFN*-γ genes in various tumors, there are few publications about their relationship with HCC. Therefore, our study aimed to explore the effects of *IFN*-γ rs2069705, rs2069727, and *MICA* rs2596542 polymorphisms on HCC, as well as influences of gene-gene interaction on the incidence of the disease.

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 NaI 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'-TAGCACTTTATGAGGATTAC-3' and 5'-AGGTAAT CCTCATAAAGTGC-3'; rs2069727: 5'-AGGTTCTGCTATGGAATGTA-3' and 5'-AAACTACATTCCATAGCAGA-3'; and rs2596542: 5'-TCGT CTCCCAAAGAACAGCTAC-3' and 5'-CCAGTCTCTGGAGTCACTGTC-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 7vmin. 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 χ^2 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

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 1. Comparison of genotype and allele distributions of *IFN*-γ rs2069705, rs2069727, and *MICA* rs2596542 based on HCC.

Genotype/Allele	Case n=120 (%)	Control n=124 (%)	χ^2	*P*	OR (95% CI)
IFN-γ 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)

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 χ^2 test.

Results

HWE test

Through the χ^2 test, the genotype distributions of *IFN*-γ 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-γ and MICA and HCC

The linkage between *IFN*-γ and *MICA* polymorphisms and HCC is detailed in Table 1. Specifically, GG genotype of *IFN*-γ 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*-γ 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 of 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

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

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.

genotypes carriers had 5.56 times higher risk of developing HCC (OR=6.56, 95% CI=2.29–18.83). Therefore, an obvious increase in HCC risk existed with the interaction between polymorphisms of *IFN-γ* and *MICA* genes.

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]. Therefor, 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. Our

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

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.

References:

1. Reed JR, Eyer M, Backes WL: Functional interactions between cytochromes P450 1A2 and 2B4 require both enzymes to reside in the same phospholipid vesicle: evidence for physical complex formation. J Biol Chem, 2010; 285(12): 8942–52
2. Dienz O, Eaton SM, Bond JP et al: The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. J Exp Med, 2009; 206(1): 69–78
3. Schutte K, Bornschein J, Malfertheiner P: Hepatocellular carcinoma – epidemiological trends and risk factors. Dig Dis, 2009; 27(2): 80–92
4. Högye M, Mándi Y, Csanády MM et al: Comparison of circulating levels of interleukin-6 and tumor necrosis factor-alpha in hypertrophic cardiomyopathy and in idiopathic dilated cardiomyopathy. Am J Cardiol, 2004; 94(2): 249–51
5. Zhang PL, Levy AM, Ben-Simchon L et al: Induction of neuronal and myelin-related gene expression by IL-6-receptor/IL-6: a study on embryonic dorsal root ganglia cells and isolated Schwann cells. Exp Neurol, 2007; 208(2): 285–96
6. Liu SP, Li YS, Lee CM et al: Higher susceptibility to aflatoxin B(1)-related hepatocellular carcinoma in glycine N-methyltransferase knockout mice. Int J Cancer, 2011; 128(3): 511–23
7. Budhu A, Wang XW: The role of cytokines in hepatocellular carcinoma. J Leukoc Biol, 2006; 80(6): 1197–213
8. Pyo CW, Hur SS, Kim YK et al: Polymorphisms of IL-1B, IL-1RN, IL-2, IL-4, IL-6, IL-10, and IFN-gamma genes in the Korean population. Hum Immunol, 2003; 64(10): 979–89
9. Chevillard C, Henri S, Stefani F et al: Two new polymorphisms in the human interferon gamma (IFN-gamma) promoter. Eur J Immunogenet, 2002; 29(1): 53–56
10. Henri S, Stefani F, Parzy D et al: Description of three new polymorphisms in the intronic and 3'UTR regions of the human interferon gamma gene. Genes Immun, 2002; 3(1): 1–4
11. Bahram S, Bresnahan M, Geraghty DE, Spies T: A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci USA, 1994; 91(14): 6259–63
12. Groh V, Bahram S, Bauer S et al: Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc Natl Acad Sci USA, 1996; 93(22): 12445–50
13. Bauer S, Groh V, Wu J et al: Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science, 1999; 285(5428): 727–29
14. Raffaghello L, Prigione I, Airoldi I et al: Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. Neoplasia, 2004; 6(5): 558–68
15. Yu MW, Chang HC, Liaw YF et al: Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. J Natl Cancer Inst, 2000; 92(14): 1159–64
16. Bataller R, North KE, Brenner AD: Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. Hepatology, 2003; 37(3): 493–503
17. Kohga K, Takehara T, Tatsumi T et al: Serum levels of soluble major histocompatibility complex (MHC) class I-related chain A in patients with chronic liver diseases and changes during transcatheter arterial embolization for hepatocellular carcinoma. Cancer Sci, 2008; 99(8): 1643–49
18. Lavado-Valenzuela R, Benavides M, Carabantes F, Alonso A, Caballero A: MHC class I chain-related gene A transmembrane polymorphism in Spanish women with breast cancer. Tissue Antigens, 2009; 74(1): 46–49
19. Hov JR, Lleo A, Selmi C et al: Genetic associations in Italian primary sclerosing cholangitis: heterogeneity across Europe defines a critical role for HLA-C. J Hepatol, 2010; 52(5): 712–17
20. Holdenrieder S, Eichhorn P, Beuers U et al: Soluble NKG2D ligands in hepatic autoimmune diseases and in benign diseases involved in marker metabolism. Anticancer Res, 2007; 27(4A): 2041–45
21. Hoshida Y, Fuchs BC, Tanabe KK: Genomic risk of hepatitis C-related hepatocellular carcinoma. J Hepatol, 2012; 56(3): 729–30
22. Chen A, Shen Y, Xia M et al: Expression of the nonclassical HLA class Iand MICA/B molecules in human hepatocellular carcinoma. Neoplasma, 2011; 58(5): 371–76
23. Miller RC, Martenson JA, Sargent DJ et al: Acute treatment-related diarrhea during postoperative adjuvant therapy for high-risk rectal carcinoma. Int J Radiat Oncol Biol Phys, 1998; 41(3): 593–98
24. Rossouw M, Nel HJ, Cooke GS et al: Association between tuberculosis and a polymorphic NFkappaB binding site in the interferon gamma gene. Lancet, 2003; 361(9372): 1871–72
25. Ben-Ari Z, Mor E, Papo O et al: Cytokine gene polymorphisms in patients infected with hepatitis B virus. Am J Gastroenterol, 2003; 98(1): 144–50
26. Matos GI, Covas Cde J, Bittar Rde C et al: IFNG +874T/A polymorphism is not associated with American tegumentary leishmaniasis susceptibility but can influence *Leishmania* induced IFN-gamma production. BMC Infect Dis, 2007; 7: 33

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]