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Original Article

Association of *NQO1* and *ESR1* polymorphisms with hepatocellular carcinoma susceptibility

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Abstract: Aims: With the purpose of investigating the association between *NQO1* and *ESR1* polymorphisms and the susceptibility to hepatocellular carcinoma (HCC), and providing scientific basis for the prevention and treatment of the disease, this case-control study was designed. Methods: The genotypes of *NQO1*, *ESR1* gene polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in 97 HCC patients and 72 healthy persons. The χ^2 test was used to calculate the differences of genotypes, alleles in gene polymorphisms and the other indexes between the case and control groups as well as Hardy-Weinberg equilibrium (HWE) in controls. The relative risk intensity was represented by odds ratio (OR) with 95% confidence interval (95% CI). Results: The genotype and allele distributions in both of *NQO1* rs10517 and *ESR1* rs9322354 in two groups had statistical significant differences ($P<0.05$). Not only HBsAg state affects the onset of HCC but also it existed the interaction with *NQO1* rs10517 and *ESR1* rs9322354 polymorphims to regulate HCC susceptibility ($P<0.05$). What's more, in present study, *NQO1* rs10517 and *ESR1* rs9322354 were proved the interaction and the mutations of them occurred into one individual simultaneously, which significantly increased the susceptibility of HCC (OR=4.396, 95% CI=1.097-17.621). Conclusion: *NQO1* and *ESR1* polymorphisms are susceptible factors for HCC. The interaction between them increases the onset risk of HCC.

Keywords: *ESR1*, *NQO1*, polymorphism, interaction, hepatocellular carcinoma (HCC), hepatitis B surface antigen (HBsAg)

Introduction

Hepatocellular carcinoma (HCC) is a leading cause of death from malignant tumors in the world [1]. Since the 1990s, the morbidity of HCC has been the second highest among the malignant tumors of our country, 130,000 people die from it annually [2, 3]. The occurrence of HCC is a multifactorial complex biological process with the involvement of environmental and genetic factors. A number of cases indicate the development of HCC is related to chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection, the latter may induce HCC by direct or indirect mechanisms [4]. Certainly, genetic background may determine the risk of individuals developing HCC to a large extent [5-8]. Looking for the susceptibility genes to HCC have always been attached great importance and single nucleotide polymorphism (SNP) in gene is a well tool.

Estrogen receptor1 (ESR1) is a transcription factor that is activated by the type I ligand [9, 10]. Estrogen and its receptor have been reported to be closely associated with HCC [11-13]. NAD(P)H: quinone oxidoreductase 1 (*NQO1*) is a kind of flavoenzyme and plays an important role in the detoxification metabolism process of the body [14-17]. The genetic mutations of *ESR1* and *NQO1* genes may encode the abnormal protein to result in the activity discrepancies of ESR1 transcription factor and NQO1 enzyme, which finally affects the onset of many tumors [18-22]. However, the reports about the relationship between *ESR1*, *NQO1* polymorphisms and HCC susceptibility are few.

In the process of carcinogenesis, the roles of *ESR1* and *NQO1*, the mechanism of inducing cancer, and the changes of the disease in the treatment process have become the hot spots for researchers to delve into in recent years. The present study set a group of HCC patients

Table 1. The detailed information of *NQO1* and *ESR1* primers

Locus	PCR primer	Length	Restriction enzyme
rs1800566	F: 5'-TCCTCAGAGTGGCATTCTGC-3'	211 bp	Hinf I
	R: 5'-TCTCCTCATCCTGTACCTCT-3'		
rs10517	F: 5'-ACCTGGCCCTTGCAATCTT-3'	379 bp	Nco I
	R: 5'-GCACCACAAGAGGGCAGT-3'		
rs9322354	F: 5'-GCCCTGTTTTTATTCCCAGAAC-3	479 bp	MseI
	R: 5'-ATTCATCATGCGGAACCG-3'		
rs9340772	F: 5'-TCCAGCAGCGACGACA-3'	383 bp	BsaWI
	R: 5'-GCAGAAGGCTCAGAAACC-3'		

Table 2. Comparison of clinical features between the case and control groups

Clinical feature	Case	Control
Male/Female	70/27	49/23
Age	48.73±11.08	48.86±11.19
Smoking		
Yes/No	46/51	26/46
Drinking		
Yes/No	32/65	20/52
HBsAg		
Positive/Negative	57/40	14/58*

Note: "*"stands for statistically significant difference between groups ($P<0.05$).

and normal persons as the subjects to be researched the association of *ESR1*, *NQO1* polymorphisms and other related risk factors with the occurrence of HCC. We hoped that the roles of *ESR1* and *NQO1* polymorphisms in the occurrence and development of HCC could be clarified and provided some clues for explaining the ethology of HCC through this study. In this case, we can lay a scientific foundation for prevention and treatment of HCC.

Materials and methods

Cases and controls

97 HCC cases diagnosed by pathology were enrolled into the case group. The cases were in treatment in Chinese PLA General Hospital during from October, 2011 to May, 2013. They had an age range of 21-67, including 70 males and 27 females. Patients with the following conditions were excluded: (1) with severe heart or kidney diseases as well as hypertension and diabetes; (2) with liver relative disease; (3) with autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis; (4) with autoimmune and metabolic diseases; (5) with HIV infection; (6) with antiviral treatments such as interferon and lamivudine.

The selection of the controls was frequency-matched with the cases by age and sex. Therefore, the homochronous 72 healthy controls without liver diseases were recruited into the control group from the same region with the cases, consisted of 49 males and 23 females. Their age was from 17 to 64 years old. The subjects were all Chinese Han population but they were no relationship each other. This case-control design was supported by the Research Ethics Committee of Chinese PLA General Hospital and meanwhile, all subjects and their family were informed the whole process of this study and signed the informed consent.

Clinical data collection

The subjects agreed to be surveyed by our investigators using unified questionnaires. The content of the survey included sex, age, Hepatitis B surface antigen (HBsAg) state, smoking, and drinking status.

DNA extraction

2 ml peripheral blood from every subject was collected and put into the corresponding blood collection tube, then stored at -80°C refrigerator.

The peripheral blood DNA was extracted by the method of phenol-chloroform extraction and then preserved at -20°C for standby application.

Genotyping of ESR1 and NQO1 polymorphisms

In this study, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotypes of *ESR1* and *NQO1* polymorphisms in every study subject. The primers design and synthesis were finished by Shanghai Sangon Biotech Co. Ltd and the sequences were listed in **Table 1**. The PCR reaction system consisted of 2.5 μl 10× buffer solution (including 1.5 mM Mg^{2+}), 0.5 μl dNTP

Table 3. Distributions of *NQO1* alleles and genotypes in case and control groups

SNPs	Group	Allele		*P*	Genotype			*P*	P_{HWE}
rs1800566		C	T		CC	CT	TT		
	Case	99 (51.0)	95 (49.0)		27 (27.8)	45 (46.4)	25 (25.8)		
	Control	76 (52.8)	68 (47.2)	0.826	19 (26.4)	38 (52.8)	15 (20.8)	0.670	0.618
rs10517		C	T		CC	CT	TT		
	Case	66 (34.0)	128 (66.0)		10 (10.3)	46 (47.4)	41 (42.3)		
	Control	68 (47.2)	76 (52.8)	0.018	12 (16.7)	44 (61.1)	16 (22.2)	0.022	0.055

Table 4. Distributions of *ESR1* alleles and genotypes in case and control groups

SNPs	Group	Allele		*P*	Genotype			*P*	P_{HWE}
rs9340772		C	T		CC	CT	TT		
	Case	193 (99.5)	1 (0.5)		96 (99.0)	1 (1.0)	0		
	Control	143 (99.3)	1 (0.7)	-	71 (98.6)	1 (1.4)	0	-	0.953
rs9322354		A	G		AA	AG	GG		
	Case	96 (49.5)	98 (50.5)		29 (29.9)	38 (39.2)	30 (30.9)		
	Control	92 (63.9)	52 (36.1)	0.011	31 (43.1)	30 (41.7)	11 (15.3)	0.044	0.411

Table 5. *NQO1* genotypes and alleles comparison in different HBsAg state of the case group

SNPs	HBsAg	Allele		*P*	Genotype			*P*
rs1800566		C	T		CC	CT	TT	
	Positive	56	58		14	28	15	
	Negative	43	37	0.561	13	17	10	0.680
rs10517		C	T		CC	CT	TT	
	Positive	30	84		3	24	30	
	Negative	36	44	0.006	7	22	11	0.021

Table 6. *ESR1* genotypes and alleles comparison in different HBsAg state of the case group

SNPs	HBsAg	Allele		*P*	Genotype			*P*
rs9322354		A	G		AA	AG	GG	
	Positive	46	68		11	24	22	
	Negative	50	30	0.003	18	14	8	0.017

(10 mmol/L), 1.0 μl 5 U Taq enzyme (Promega company, USA), 2.0 μl primers (20 μmol/L, forward and reverse 1.0 μl each), 1.0 μl DNA template (500 ng) and 18 μl ddH_2O. The amplification process was: 94°C initial denaturation for 2 min; 35 cycles of 94°C denaturation for 40 s, annealing for 40 s (the temperature was as **Table 1**), 72°C extension for 40 s; at last 72°C extension for 10 min. PEBi-osystems company from USA took charge of the amplification reaction. The PCR products were purified by using Multiscreen-PCR purification plates from Millipore Company of USA.

After purification, the PCR products were digested by restriction enzyme. Each enzyme reaction system was a total of 20 μl solution, which contained 10 μl PCR products, 1.0 μl 5 U endonuclease, 2.0 μl 10× loading buffer solution, the rest volume filled with ddH_2O. The reaction system was performed in water bath for 12 h. After digestion, 2% agarose gel electrophoresis was conducted and the genotypes of the four polymorphisms were determined according to the electrophoretic bands.

Statistical analysis

The distribution differences of genotypes, alleles and the other indexes were compared by χ^2 test which was to verify whether the genotype frequencies were in accordance with Hardy-Weinberg equilibrium (HWE) in the control group. The correlation between gene polymorphisms and HCC risk was displayed by odds ratio (OR) and 95% confidence interval (95% CI). All of the statistical analyses were systemized by PASW Statistics 18.0 software and $P<0.05$ was a significant difference.

Results

Clinical characteristics comparison between case and control groups

The analyses of sex, age, smoking, drinking, and HBsAg status in the two groups indicated

Table 7. The interaction analysis of *NQO1* and *ESR1* polymorphisms

NQO1/SNP	*ESR1/SNP*	Case	Control	OR (95% CI)	*P*
rs1800566	rs9322354				
+	+	17	13	Reference	-
+	-	10	6	1.275 (0.368-4.419)	0.702
-	+	12	18	0.510 (0.183-1.424)	0.196
-	-	58	35	1.267 (0.550-2.921)	0.578
rs10517	rs9322354				
+	+	3	8	Reference	-
+	-	7	4	4.667 (0.765-28.466)	0.087
-	+	26	23	3.014 (0.714-12.731)	0.122
-	-	61	37	4.396 (1.097-17.621)	0.025

Note: "+" stands for wild type; "-" stands for mutant type.

that the frequency of positive HBsAg was significantly higher in case group than in control group ($P<0.05$). No clear correlation of sex, age, smoking, and drinking status between the two groups was observed ($P>0.05$) (**Table 2**).

Correlation between NQO1 and ESR1 polymorphisms and HCC

The genotypes distributions of *NQO1*, *ESR1* polymorphisms were detected to be consistent with HWE, the results was showed in **Tables 3**, **4**, which demonstrated that our study population was eligible and possessed the representativeness.

The results were displayed in **Table 3**, the genotype and allele distributions of *NQO1* rs1800566 between two groups had no statistically significant differences ($P>0.05$). The frequencies of CC, CT, and TT genotype of rs10517 were respectively 10.3%, 47.4%, 42.3% and 16.7%, 61.1%, 22.2% in case and control groups. The TT genotype was more frequent in case group than in control group, and the difference was statistically significant ($P<0.05$). The C and T allele frequencies of rs10517 were respectively 34.0%, 66.0% and 47.2%, 52.8% in cases and controls. The statistically significant difference was found in allele distributions of two groups ($P<0.05$).

The mutation frequencies of *ESR1* rs9340772 genotypes and alleles were too low to be analyzed. The AA, AG, GG genotype frequencies of rs9322354 were respectively 29.9%, 39.2%, 30.9% and 43.1%, 41.7%, 15.3% in cases and controls. The GG genotype frequency of the case group was significantly higher than that of the control group, with statistically significant level at $P<0.05$. The A and G alleles frequencies of rs9322354 were 49.5%, 50.5% and 63.9%, 36.1% in case and control groups respectively. The allele frequencies had statistically significant difference between two groups ($P<0.05$). The results were shown in **Table 4**.

Correlation of NQO1 and ESR1 polymorphisms with HBsAg state

The genotype and allele distributions of *NQO1* rs1800566 between the cases with positive HBsAg and negative HBsAg had no statistically significant difference ($P>0.05$), but significant differences in genotype and allele distributions of rs10517 were detected between the above two kinds of patients ($P<0.05$) (**Table 5**).

In **Table 6**, the association between the genotype and allele distributions of *ESR1* rs9322354 polymorphism and HBsAg state in HCC patients was displayed, we can see that the results were certain ($P<0.05$).

Interaction analysis between NQO1 and ESR1 polymorphisms in case and control groups

The *NQO1* rs1800566 and rs10517 polymorphisms were respectively analyzed the interactions with *ESR1* rs9322354 polymorphism. We found that the simultaneous mutation of *NQO1* rs10517 and *ESR1* rs9322354 remarkably increased the onset risk of HCC (OR=4.396, 95% CI=1.097-17.621), which suggested the interaction might exist between two polymorphisms (**Table 7**).

Discussion

Human *NQO1* is located on chromosome 16q22 and encodes the NQO1 enzyme. The non-synonymous polymorphisms at the coding domain and phenotypic modification of *NQO1* can change the amino acids sequences, reduce or even vanish NQO1 enzyme activities, weaken the NQO1 detoxification, which influences the functions of specific proto-oncogenes and tumor suppressor genes. As a result, the pos-

sibilities of individuals suffering from cancer are increased [23-25]. Animal experiments prove that the carcinogenic agent DMBA in the epithelium of mice decreases *NQO1* activity and meanwhile, significantly increases the rate of tumor formation, tumor size and the proportion of proliferating cells. These results laterally confirm the protective effects of *NQO1* as the important quinone detoxification enzyme on the occurrence of tumors [26-28]. Some other researchers have also confirmed the correlation of *NQO1* polymorphisms with a variety of tumors development.

Estrogen has a strong inhibitory effect on the occurrence and development of HCC. The reduction of ESR expression in HCC patients indicates that ESRs may be the protective receptors against HCC. Some scholars have found that the incidence of HCC has significant gender difference. Not only the natural survival rate but also the postoperative survival rate of premenopausal females are lower than that of the males, which may be due to the estrogen level of the individuals [29].

In present study, firstly, some clinical characteristics were tested the role in HCC development and progression. We found that age, gender, smoking, alcohol consumption were no significantly associated with HCC, but the HBsAg state had obvious difference between the cases and controls. However, in previous study, alcohol consumption and even gender involved in the generation of HCC [29, 30]. The different results may be caused the small sample size in our study and the different regions, races. *NQO1* rs1800566, rs10517 polymorphisms and *ESR1* rs9340772, rs9322354 polymorphisms were selected to explore the correlation to HCC susceptibility. Neither genotype nor allele distributions of *NQO1* rs1800566 and *ESR1* rs9340772 between two groups had statistically significant differences and the results showed that these two polymorphisms may not affect the function of *NQO1*, *ESR1* or they don't play the independent roles in HCC occurrence. Differently, another two polymorphisms rs-10517, rs9322354 of these two genes were associated with the onset risk of HCC and may be the susceptible factors to HCC. The interaction analysis of these two polymorphisms discovered that the simultaneous occurrence of their mutations increased the risk of HCC development. The stratified analysis on HBsAg state in case group showed that the genotype and allele distributions of rs10517 and rs9322354 polymorphisms in positive and negative HBsAg groups existed significant differences, which suggested that HBsAg was a biomarker and possessed the genotype bias based on HCC.

This article analyzes the *NQO1* and *ESR1* polymorphisms in HCC patient and healthy people, which provides molecular genetic basis for further investigating the ethology and pathology of HCC. However, as we all known, the types and frequencies of genotypes and alleles in *NQO1* and *ESR1* polymorphisms are correlated with race and region. Moreover, different lifestyles and environmental carcinogens of different racial groups have a vital influence on the occurrence of HCC, besides, our study population is small. Therefore, It is necessary to conduct further case-control studies with multinational and multi-population are as so to confirm the correlation of *NQO1* and *ESR1* polymorphisms with the HCC genetic susceptibility.

Disclosure of conflict of interest

None.

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References

[1] El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology 2012; 142: 1264-1273 e1261.

[2] Herszenyi L and Tulassay Z. Epidemiology of gastrointestinal and liver tumors. Eur Rev Med Pharmacol Sci 2010; 14: 249-258.

[3] Chen JG and Zhang SW. Liver cancer epidemic in China: past, present and future. Semin Cancer Biol 2011; 21: 59-69.

[4] Pollicino T, Saitta C and Raimondo G. Hepatocellular carcinoma: the point of view of the hepatitis B virus. Carcinogenesis 2011; 32: 1122-1132.

[5] Dongiovanni P, Donati B, Fares R, Lombardi R, Mancina RM, Romeo S and Valenti L. PNPLA3 I148M polymorphism and progressive liver disease. World J Gastroenterol 2013; 19: 6969-6978.

[6] Gao J, Xu HL, Gao S, Zhang W, Tan YT, Rothman N, Purdue M, Gao YT, Zheng W, Shu XO and Xiang YB. Genetic polymorphism of NFKB1 and NFKBIA genes and liver cancer risk: a nested case-control study in Shanghai, China. BMJ Open 2014; 4: e004427.

[7] Li QY, Zhao NM, Wang LC, Duan HF, Ma YC, Zhang W, Zhao HW and Qin YH. Individuals having variant genotypes of cytochrome P450 2C19 are at increased risk of developing primary liver cancer in Han populations, without infection with the hepatitis virus. Tumour Biol 2014; 35: 9023-9026.

[8] Xiao Y and Ma JZ. [Study of the relationship between glutathione S-transferase genetic polymorphisms M1 and T1 and susceptibility to primary liver cancer in Chinese: a meta-analysis]. Zhonghua Gan Zang Bing Za Zhi 2012; 20: 774-779.

[9] Muramatsu M and Inoue S. Estrogen receptors: how do they control reproductive and nonreproductive functions? Biochem Biophys Res Commun 2000; 270: 1-10.

[10] Beato M and Klug J. Steroid hormone receptors: an update. Hum Reprod Update 2000; 6: 225-236.

[11] Baum JK, Bookstein JJ, Holtz F and Klein EW. Possible association between benign hepatomas and oral contraceptives. Lancet 1973; 2: 926-929.

[12] Neuberger J, Forman D, Doll R and Williams R. Oral contraceptives and hepatocellular carcinoma. Br Med J (Clin Res Ed) 1986; 292: 1355-1357.

[13] Eagon PK, Francavilla A, DiLeo A, Elm MS, Gennari L, Mazzaferro V, Colella G, Van Thiel DH and Strazl TE. Quantitation of estrogen and androgen receptors in hepatocellular carcinoma and adjacent normal human liver. Dig Dis Sci 1991; 36: 1303-1308.

[14] Chen S, Wu K and Knox R. Structure-function studies of DT-diaphorase (NQO1) and NRH: quinone oxidoreductase (NQO2). Free Radic Biol Med 2000; 29: 276-284.

[15] Warrington KH Jr, Teschendorf C, Cao L, Muzyczka N and Siemann DW. Developing VDEPT for DT-diaphorase (NQO1) using an AAV vector plasmid. Int J Radiat Oncol Biol Phys 1998; 42: 909-912.

[16] Singh S, Zahid M, Saeed M, Gaikwad NW, Meza JL, Cavalieri EL, Rogan EG and Chakravarti D. NAD(P)H: quinone oxidoreductase 1 Arg139Trp and Pro187Ser polymorphisms imbalance estrogen metabolism towards DNA adduct formation in human mammary epithelial cells. J Steroid Biochem Mol Biol 2009; 117: 56-66.

[17] Amara IE and El-Kadi AO. Transcriptional modulation of the NAD(P)H:quinone oxidoreductase 1 by mercury in human hepatoma HepG2 cells. Free Radic Biol Med 2011; 51: 1675-1685.

[18] Sun H, Deng Q, Pan Y, He B, Ying H, Chen J, Liu X and Wang S. Association between estrogen receptor 1 (ESR1) genetic variations and cancer risk: a meta-analysis. J BUON 2015; 20: 296-308.

[19] Son BH, Kim MK, Yun YM, Kim HJ, Yu JH, Ko BS, Kim H and Ahn SH. Genetic polymorphism of ESR1 rs2881766 increases breast cancer risk in Korean women. J Cancer Res Clin Oncol 2015; 141: 633-645.

[20] Sun Z, Cui Y, Pei J and Fan Z. Association between NQO1 C609T polymorphism and prostate cancer risk. Tumour Biol 2014; 35: 7993-7998.

[21] Freriksen JJ, Salomon J, Roelofs HM, Te Morsche RH, van der Stappen JW, Dura P, Witteman BJ, Lacko M and Peters WH. Genetic polymorphism 609C>T in NAD(P)H: quinone oxidoreductase 1 enhances the risk of proximal colon cancer. J Hum Genet 2014; 59: 381-386.

[22] Hu WG, Hu JJ, Cai W, Zheng MH, Zang L, Wang ZT and Zhu ZG. The NAD(P)H: quinine oxidoreductase 1 (NQO1) gene 609 C>T polymorphism is associated with gastric cancer risk: evidence from a case-control study and a meta-analysis. Asian Pac J Cancer Prev 2014; 15: 2363-2367.

[23] Park SJ, Zhao H, Spitz MR, Grossman HB and Wu X. An association between NQO1 genetic polymorphism and risk of bladder cancer. Mutat Res 2003; 536: 131-137.

[24] Saldivar SJ, Wang Y, Zhao H, Shao L, Lin J, Spitz MR and Wu X. An association between a NQO1 genetic polymorphism and risk of lung cancer. Mutat Res 2005; 582: 71-78.

[25] Siegel D and Ross D. Immunodetection of NAD(P)H: quinone oxidoreductase 1 (NQO1) in human tissues. Free Radic Biol Med 2000; 29: 246-253.

[26] Girolami F, Abbadessa G, Racca S, Spaccamiglio A, Piccione F, Dacasto M, Carletti M, Gardini G, Di Carlo F and Nebbia C. Time-dependent acetylsalicylic acid effects on liver CYP1A and antioxidant enzymes in a rat model of 7,12-dimethylbenzanthracene (DMBA)-induced mammary carcinogenesis. Toxicol Lett 2008; 181: 87-92.

[27] Talalay P and Dinkova-Kostova AT. Role of nicotinamide quinone oxidoreductase 1 (NQO1) in protection against toxicity of electrophiles and reactive oxygen intermediates. Methods Enzymol 2004; 382: 355-364.

[28] Dinkova-Kostova AT, Fahey JW and Talalay P. Chemical structures of inducers of nicotinamide quinone oxidoreductase 1 (NQO1). Methods Enzymol 2004; 382: 423-448.

[29] El-Serag HB and Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007; 132: 2557-2576.

[30] Urata Y, Yamasaki T, Saeki I, Iwai S, Kitahara M, Sawai Y, Tanaka K, Aoki T, Iwadou S, Fujita N, Nakayama Y, Maeshiro T, Takami T and Sakaida I. Clinical characteristics and prognosis of non-B non-C hepatocellular carcinoma patients with modest alcohol consumption. Hepatol Res 2015; [Epub ahead of print].