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Original Article

Effect of APE1 and XRCC1 gene polymorphism on susceptibility to hepatocellular carcinoma and sensitivity to cisplatin

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Abstract: Objective: The relationship between APE1 and XRCC1 gene polymorphism and the susceptibility to hepatocellular carcinoma (HCC) was discussed, and the effect of APE1 and XRCC1 gene polymorphism on the sensitivity of HCC to cisplatin was investigated. Method: From January 2010 to August 2014, 118 HCC patients were admitted to our hospital. 120 patients treated for non-tumor diseases during this period were recruited as controls. PCR-RFLP analyses were performed to determine the association between APE1 Asp148Glu and XRCC1 Arg194Trp polymorphism, risk of HCC, and sensitivity to cisplatin. Results: The risk of HCC in patients with Glu/Glu genotype of APE1 gene was increased by 4.510 times (95% CI: 1.235~16.472, P<0.05). Compared with Asp/Asp, the risk of cisplatin resistance in patients with Glu/Glu genotype was increased by 10.500 times (95% CI: 1.800~61.241). Compared Arg/Arg genotype, the risk of cisplatin resistance in patients with Arp/Trp genotype of XRCC1 gene was increased by 6.701 times (95% CI: 1.464~30.732, P<0.05). Conclusion: APE1 Asp148Glu polymorphism is associated with the susceptibility to HCC. APE1 Asp148Glu and XRCC1 Arg194Trp polymorphism plays a part in the cisplatin resistance of HCC cells.

Keywords: Gene polymorphism, hepatocellular carcinoma, susceptibility, sensitivity to cisplatin, cisplatin resistance

Introduction

Hepatocellular carcinoma (HCC) ranks the fifth in terms of incidence throughout the world. Patients with HCC are usually diagnosed at a late stage when the conditions are no longer suitable for surgical resection and liver transplantation [1]. Transcatheter arterial chemoembolization (TACE) is the preferred method to treat unresectable HCC. However, the efficacy of TACE is not very satisfactory, the 2-year survival and 5-year survival being 33.9% and 17.2%, respectively. Cisplatin is the major agent used in TACE, while cisplatin resistance is one factor influencing the treatment effect for HCC [2]. The variation of the apurinic/apyrimidinic endonuclease 1 (APE1) gene and the XPD gene, which are DNA damage repair genes, serves as an important molecular basis for cisplatin resistance of tumor cells [3-5]. An association has been found between APE1 gene and XPD gene polymorphism and HCC, with Arg149Trp polymorphism in X-ray repair cross complementing gene 1 (XRCC1) and Asp149Glu polymorphism in APE1 gene. The reported polymorphism can affect the activity of XRCC1 and APE1 [6, 7]. Strategy for individualized therapy should be explored based on the resistance mechanism in HCC, so as to improve the specificity and sensitivity of therapy and to reduce toxicity.

Subjects and methods

Subjects

A case-control study was designed. The case group consisted of 118 HCC patients hospitalized from January 2010 to August 2014, and the control group consisted of 120 patients treated for non-tumor diseases at our hospital. Informed consent was obtained from all subjects or their relatives.

Table 1. Comparison of the basic information of the two groups [n (%)]

Basic information		HCC group (n=118)	Control group (n=120)	P
Sex	Male	85 (72.0)	73 (60.8)	0.224
	Female	33 (28.0)	47 (39.2)	
Age	≤50 years old	78 (66.1)	65 (54.2)	0.213
	>50	40 (33.9)	55 (45.8)	
Smoking history	Yes	40 (33.9)	19 (15.8)	0.005
	No	78 (66.1)	101 (84.2)	
Drinking history	Yes	49 (41.5)	17 (14.2)	0.001
	No	69 (58.5)	103 (85.8)	

Table 2. Comparison of frequency distribution of APE1 and XRCC1 genotypes [n (%)]

Gene	Genotype	HCC group (n=118)	Control group (n=120)	P
APE1	Asp/Asp	94 (79.7)	106 (88.3)	0.013
	Asp/Glu	7 (5.9)	11 (9.2)	
	Glu/Glu	17 (14.4)	3 (2.5)	
XRCC1	Arg/Arg	55 (46.6)	58 (48.3)	0.323
	Arg/Trg	53 (44.9)	45 (37.5)	
	Trp/Trp	10 (8.5)	17 (14.2)	

After Bonferroni correction: APE1 gene: Asp/Asp vs. Asp/Glu: P=0.437; Asp/Asp vs. Glu/Glu: P=0.010; Asp/Glu vs. Glu/Glu: P=0.013.

Inclusion criteria

Inclusion criteria for the case group: (1) In accordance with the diagnostic criteria recommended by the 2001 China National Conference on the Prevention and Control of Liver Cancer [4], all patients received liver function test, routine blood test and ultrasonograhy. The level of alpha fetoprotein (AFP) was measured. For those with AFP≥200 μg/L and no space-occupying lesion found by ultrasonography, CT, MRI or angiography was performed. The confirmed cases with unresectable HCC were included; (2) No previous chemotherapy or radiotherapy for any cases; (3) Not concurrent with other malignant tumors or dysfunction of important organs.

Inclusion criteria for the control group: (1) Matched in such attributes as sex, age and place or residence (urban or rural) with those in the case group; (2) Non-tumor diseases; (3) No family history of tumors.

Information collection

The self-designed inquiry form was used to collect the basic information of the subjects, including demographic characteristics (age, sex, marital status, lifestyle, personal and family history of disease. Peripheral blood of 4 mL was collected from each subject, and 2 mL was used for biochemical indicator testing. The remaining part of the sample was anticoagulated and cryopreserved until DNA extraction.

Reagents and instruments

Reagents: DNA primers, PCR Assay Kit, restriction endonucleases, proteinase K, agarose, DNA markers (100 bp), and fluorescent dye GeneFinder (100 μL) were provided by Sangon Biotech (Beijing) Co., Ltd. Tris (hydroxymethyl) aminomethane, potassium iodide, chloroform, isoamyl alcohol, isopropyl alcohol, and 70% ethanol were provided by Xiamen Zeesan Biotech Co., Ltd.

Instruments: Centrifuge (Sigma, USA), PCR system (Thermoeycler, USA), electrophoresis gel imaging system (Beijing CBIO Bioscience & Technologies CO., Ltd.), electrophoresis apparatus and electrophoresis tank (SCIE-PLAS Biochrom, UK), UV spectrophotometer (Shanghai Mapada Instruments Co., Ltd), micropipettor (Beijing Keeven Zhuoli Precision Equipment Co., Ltd.), micro-electronic balance (Beijing Bo Yuan Cheung Tak Scientific Instrument Co., Ltd).

Experimental method

DNA extraction: Equal volume of 3% gelatin was added into 2 mL of tissue homogenate of HCC and mixed well. The tube was kept in the water bath at 37°C for 10 min. The supernatant was taken and centrifuged for 5 min at 5000 r/min. The precipitate was placed into the tube and added with TES buffer and 10 drops of 10% SDS and mixed well. Then 2 mL of Tris-phenol was added with mixing, and the solution was centrifuged at 5000 r/min for 5 min. The supernatant was transferred to another centrifuge tube, added with equal volume of chloroform and centrifuged at 5000 r/min for 5 min. The obtained supernatant was transferred to

Table 3. Association between APE1 and XRCC1 gene polymorphism and susceptibility to HCC [n (%)]

Gene	Genotype	HCC group (n=118)	Control group (n=120)	OR (95% CI)	P
APE1	Asp/Asp	94 (79.7)	106 (88.3)	1	
	Asp/Glu	12 (10.2)	11 (9.2)	1.230 (0.518~2.918)	0.638
	Glu/Glu	12 (10.2)	3 (2.5)	4.510 (1.235~16.472)	0.022
XRCC1	Arg/Arg	55 (46.6)	58 (48.3)	1	
	Arg/Trg	53 (44.9)	45 (37.5)	1.242 (0.722~2.136)	0.433
	Trp/Trp	10 (8.5)	17 (14.2)	0.620 (0.261~1.471)	0.279

Table 4. Association between APE1 and XRCC1 gene polymorphism and sensitivity to cisplatin [n (%)]

Gene	Gene	Insensitivity group (n=18)	Sensitivity group (n=30)	OR (95% CI)	P
APE1	Asp/Asp	8 (44.4)	24 (80.0)	1	
	Asp/Glu	3 (16.7)	4 (13.3)	2.250 (0.412~12.284)	0.349
	Glu/Glu	7 (38.9)	2 (6.7)	10.500 (1.800~61.241)	0.009
XRCC1	Arg/Arg	6 (33.3)	23 (76.7)	1	
	Arg/Trg	7 (38.9)	4 (13.3)	6.708 (1.464~30.732)	0.014
	Trp/Trp	5 (27.8)	3 (10.0)	6.388 (1.178~34.623)	0.032

another tube, into which 2.5 times volume of anhydrous alcohol was added to cause DNA to precipitate out. The precipitate was washed with 70% ethanol for twice and left to volatize. After the addition of 200 μL TE, the sample was preserved at -20°C.

PCR reaction system: PCR reaction system consisted of 10×PCR buffer 2.5 μL, primer 1 and 2 1.0 μL each, DNA template 1 μL, 2 U/μL Tap enzyme, dNTPs (2 mmol) 2 μL. The total volume was 25 μL with the addition of ddH_2O.

PCR conditions: pre-denaturation at 95°C for 5 min, at 90°C for 10 s, and at 56°C for 40 s, 32 cycles, 1 cycle of final extension at 72°C.

Restriction fragment length polymorphism (RFLP) analysis: The 25 μL RFLP analysis system consisted of 10×buffer 2.5 μL, 10 U/μL Aat II or ApaL I 1.0 μL, PCR product 10 μL, and ddH_2O 2.5 μL.

Chemotherapy and sensitivity evaluation of chemotherapy [4]

TACE was performed using cisplatin (PDD) in all 118 HCC cases, and 30 cases completed at least twice of TACE at week 6 and 12, respectively. The efficacy was evaluated according to the Response Evaluation Criteria in Solid Tumors proposed by WHO. Complete remission (CR) was defined as complete disappearance of measurable loci for 4 weeks; partial remission (PR), a decrease of the sum of the largest diameter of locus and the product of largest vertical and transverse diameter >50% for at least 4 weeks; stable disease (SD), a decrease of the sum of the largest diameter of locus and the product of largest vertical and transverse diameter <50%, or an increase >25%, or with the appearance of new loci. Cases achieving CR and PR were classified as the sensitivity group, and those achieving SD and PD as the insensitivity group.

Statistical process

The count data were expressed as ratios or proportions and analyzed by X^2 test. Non-conditional logistic regression was performed to detect the association between SNP, susceptibility to HCC, and cisplatin resistance. All statistical analyses were performed using SPSS 17.0 software, and $P<0.05$ was considered as statistically significant.

Results

Comparison of the basic information of the two groups: As shown by χ^2 test, the HCC group and the control group did not show significant differences in terms of sex, age, marital status and occupation. The two groups were matched in these attributes. However, there was a significant difference in the proportion of cases who

had drinking and smoking habits. This showed that drinking and smoking were the risk factors of HCC. The distribution of genotypes of the two genes obeyed the Hardy-Weinberg law, and the control subjects were representative of the source population (**Table 1**).

Results of RELP analysis

T→G mutation of APE1 Asp148 Glu gene was recognized by the restriction endonucleases, and one 569 bp fragment of wild-type homozygote TT (Asp/Asp) was obtained. The mutant-type homozygote GG (Glu/Glu) was cleaved into 2 fragments with the length of 187 bp and 382 bp, respectively. The mutant-type heterozygote TG (Asp/Glu) was cleaved into 3 fragments with the length of 324 bp, 187 bp and 511 bp, respectively.

C→T mutation of XRCC1 Arg194Trp gene was recognized by the restriction endonucleases, and one 511 bp fragment of wild-type homozygote CC (Arg/Arg) was obtained. The mutant-type homozygote TT (Trp/Trp) was cleaved into 2 fragments with the length of 324 bp and 187 bp, respectively. The mutant-type heterozygote CT (Arg/Trp) was cleaved into 3 fragments with the length of 324 bp, 187 bp and 155 bp, respectively.

Comparison of frequency distribution of APE1 and XRCC1 alleles and genotypes

The distribution frequencies of the genotypes of APE1 ASP148Glu and XRCC1 Arg194Trp gene were compared between the two groups. The distribution frequency of Glu/Glu genotype in HCC group was obviously higher than that of the control ($P<0.05$). The genotypes of XRCC1 Arg194Trp gene did not show significant differences in distribution frequency between the two groups ($P>0.05$). See **Table 2**.

Association between APE1 and XRCC1 gene polymorphism and susceptibility to HCC

Non-conditional logistic regression was performed with Asp/Asp and Arg/Arg as reference, and the analysis results are shown in **Table 3**. The cases with Glu/Glu genotype of APE1 gene had an increase in the risk of HCC by 4.510 times (95% CI: 1.235~16.472). However, there was no obvious difference between those with Asp/Glu and Asp/Asp genotype. The 3 genotypes of XRCC1 Arg194Trp gene, namely, Arg/Arg, Arg/Trg and Trp/Trp, did not lead to significant differences in the risk of HCC.

Association between APE1 and XRCC1 gene polymorphism and sensitivity to cisplatin

All 118 HCC cases were treated by TACE using PDD, and 48 cases received TACE for at least twice. Among them 30 cases (62.5%) were sensitive to chemotherapy, and 18 cases (37.5%) were insensitive.

Non-conditional logistic regression was performed using Asp/Asp and Arg/Arg as reference, and the relationship between APE1 and XRCC1 gene polymorphism and cisplatin sensitivity was analyzed (see **Table 4**). HCC cases with Glu/Glu genotype of APE1 gene were more likely to develop cisplatin resistance than those with Asp/Asp genotype, showing an increase in the risk by 10.500 times (95% CI: 1.800~61.241). The HCC cases with Asp/Glu and Asp/Asp genotype did not show differences in sensitivity to cisplatin. However, those with Arg/Trp or Trp/Trp genotype of XRCC1 gene showed an increase in the risk of cisplatin resistance by 6.708 times (95% CI: 1.464~30.732) and 6.388 times (95% CI: 1.178~34.623).

Discussion

HCC is one of the most common malignant tumors in China. HCC mortality rate of China ranks the first in the world, which means a great threat to people's health. The prevention and control of HCC is not only a much studied subject, but also a key issue of China's health policy [8]. Compared with other malignant tumors, HCC is featured by insidious onset and rapid progression in which many influence factors are of concern. Many patients are diagnosed at a late stage, and the clinical outcomes are usually poor [9-11]. Hence it is very important to identify the high-risk genetic features of HCC.

Single nucleotide polymorphism (SNP) is a common form of polymorphism in human genetics. DNA sequence polymorphism induced by single nucleotide variation can lead to variability of susceptibility to malignant tumors [12]. Some studies showed that SNP of XRCC1 and APE1 gene is related to the susceptibility to tumors [13, 14]. According to Shen et al. [15], compared with wild-type homozygote genotype

APE1-148 Asp/Asp, the mutant homozygote genotype APE1-148 Glu/Glu is the high-risk factor of lung cancer. However, this finding is still disputed. Other studies indicate that XRCC1 SNP has a close association with lung cancer [16], esophageal cancer [17], breast cancer [18], bladder cancer [19], gastric cancer [20] and rectal cancer [21]. There are very few studies on the relationship between APE1 and XRCC1 gene polymorphism and susceptibility to HCC, and no conclusive evidence has been known.

TACE is the preferred method to treat unresectable HCC with the use of cisplatin. The sensitivity to cisplatin has a direct impact on the efficacy of TACE, and cisplatin resistance is the major reason for the high mortality rate of HCC. However, the mechanism of cisplatin resistance in HCC remains to be further clarified. Some scholars propose the DNA repair mechanism as one source of resistance. If DNA damage is repaired immediately, cisplatin resistance is likely to occur. APE1 is the key rate-limiting enzyme in base excision repair. Besides repairing the DNA damage induced by oxidants, APE1 is also a redox actor regulating the DNA-binding activity of the transcriptional factors. This may be one mechanism for the influence of DNA repair gene polymorphism on sensitivity to cisplatin in HCC.

The association between APE1 ASP148Glu and XRCC1 Arg194Trp polymorphism and susceptibility to HCC was studied. PCR and RFLP analyses were performed to clarify the relationship between APE1 ASP148Glu and XRCC1 Arg-194Trp polymorphism, susceptibility to HCC, and sensitivity of cisplatin. The possible mechanism of cisplatin resistance in HCC was proposed. Analysis results showed that more cases in HCC group had a drinking and smoking history than in the control group ($P<0.05$). It was implied that drinking and smoking were the risk factors of HCC, as agreed by many other studies. The risk of HCC in patients with Glu/Glu genotype of APE1 ASP148Glu gene was higher by 4.510 times. But statistically significant differences were not seen between other genotypes ($P>0.05$). The reason may be that the patients with Glu/Glu genotype have a weakened DNA repair ability and hence an increased susceptibility to HCC. In contrast, the genotypes of XRCC1 Arg194Trp gene were not associated with a statistically significant difference in the risk of HCC ($P>0.05$). This finding was consistent with the previous results.

We also found that the patients with Glu/Glu genotype of APE1 Asp148Glu gene were more prone to cisplatin resistance than those with Asp/Asp genotype, showing an increase in the risk by 10.500 times (95% CI: 1.800~61.241). The patients with Arg/Trp genotype of XRCC1 Arg194Trp gene had an increase in the risk of cisplatin resistance by 6.708 times (95% CI: 1.464~30.732). These results indicated that APE1 and XRCC1 gene may be involved in the occurrence and progression of HCC. The oxidation-reduction capacities and DNA repair function possessed by the two genes have a direct connection with the cisplatin resistance of tumor cells. Gene therapy targeting at the drug resistance-related genes may improve the sensitivity to cisplatin in HCC and hence the treatment effect. More comprehensive consideration is needed for efficacy evaluation in HCC to account for the impact of different factors. The findings need to be corroborated by large-sample prospective study as the paper has a limited sample size and some baseline information is lost during follow-up.

Disclosure of conflict of interest

None.

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