Association of ERCC5 Genetic Polymorphisms With Cirrhosis and Liver Cancer

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Abstract

Introduction: To explore association of excision repair cross-complementing 5 (ERCC5) genetic polymorphisms with cirrhosis and liver cancer. Methods: A total of 365 patients were enrolled, including control group (n = 133), cirrhosis group (n = 122), and liver cancer group (n = 110). The genotyping of ERCC5 rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 was measured by using MassARRAY iPLEX technology. Results: There were no significant differences in gender and drinking among the 3 groups (P > .05). There were significant differences among the 3 groups in both age-group ≤60 and >60 subgroup patients. Locus rs2016073 was significantly different among 3 groups, and genotype GG (n = 0) was not observed in liver cancer group. As for locus rs751402, there were significant differences among 3 groups, and genotype AA (n = 0) was not observed in liver cancer group. As for locus rs2094258, there were no significant differences among 3 groups. Locus rs2296147 showed no significant differences among 3 groups (P > .05), but genotype CC was not observed in liver cancer group (n = 0). As for locus rs2296148, there were significant differences among 3 groups, and genotype TC (n = 0) was not observed in cirrhosis group. Regression analysis found locus rs751402 had significant difference between control group and cirrhosis group, patients with genotype AA and genotype GG were more likely to have cirrhosis than those with genotype GA. Conclusion: Our study suggested that genotype AA, genotype GG of ERCC5 locus rs751402, and genotype TC of locus rs2296148 may be important targets for cirrhosis, while ERCC5 polymorphisms (rs2016073 and ERCC5 polymorphisms, rs2016073 with genotype GG, and rs751402 with genotype AA) may be potential markers for liver cancer.

Keywords
ERCC5, genetic polymorphism, cirrhosis, liver cancer

Abbreviations
OR, odds ratio; SNP, single nucleotide polymorphisms.

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Introduction

Liver cancer ranks the sixth in cancer incidence and the second in tumor-related mortality worldwide, with over half of the new cases and deaths occur in China.1 Cirrhosis is a chronic liver disease and it can advance to liver cancer.2 Liver cancer is an often fatal malignant tumor with a high recurrence rate and chemoresistance.3 The relationship between time interval from diagnosis to treatment and survival status of patients with early-stage liver cancer was explored.4 However, further mechanisms of cirrhosis and liver cancer were still ambiguous.

Excision repair cross-complementing (ERCC) genes, key components of the nucleotide excision repair pathway, are regarded as crucial factors for DNA repair capacity.5 Excision repair cross-complementing 5 shows an effect on regulating

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DNA excision repair, and DNA repair capacity may be changed by its functional single nucleotide polymorphisms (SNPs), which may contribute to cancer risk. Many early studies have found ERCC5 polymorphisms to be a potential marker for a variety of cancers. Individuals with the inherited ERCC rs751402 CC genotype may experience significant protection against hepatocellular carcinoma, whereas individuals with T alleles appear to be exposed to higher risk. The expression of ERCC5 protein was significantly increased in tumor tissues compared with paracancerous tissues, and high expression of ERCC5 predicted a poor prognosis in hepatocellular carcinoma. However, there are very few reports about association of ERCC5 gene polymorphisms with cirrhosis and liver cancer. Whether ERCC5 polymorphisms could be used as potential marker for liver cancer was still unknown.

Therefore, this study was designed to explore association of ERCC5 genetic polymorphisms with cirrhosis and liver cancer. The association of ERCC5 gene polymorphisms (rs2016073, rs751402, rs2094258, rs2296147, and rs2296148) with cirrhosis and liver cancer was explored by MassARRAY iPLEX technology in this study.

Table 1. The PCR Primers.

| Genotyping | Primers |
|------------|---------|
| rs2016073  | ACGTTGGATGCTCCTTTGAAAAGGCTTATC(2nd-PCRP) | ACGTTGGATGAACAGAAGGCTTTAGG(1st-PCRP) |
| rs751402   | ACGTTGGATGTAAGGAGGGCTTTAGG(1st-PCRP) |
| rs2094258  | ACGTTGGATGAAAAGCCAGAAGGCTTTAGG(1st-PCRP) |
| rs2296147  | ACGTTGGATGCAACTGTTTCCCTCTATCTTCGTA(2nd-PCRP) |
| rs2296148  | ACGTTGGATGCAACTGTTTCCCTCTATCTTCGTA(2nd-PCRP) |
| rs2296148  | ACGTTGGATGCAACTGTTTCCCTCTATCTTCGTA(2nd-PCRP) |

Material and Methods

Patients

This is a prospective, single center, observational study. In this study, 365 patients were enrolled from the Gansu Provincial Hospital between October 2015 and December 2018. All patients were classified into 3 groups: control group (n = 133), cirrhosis group (n = 122), and liver cancer group (n = 110). The basic medical data of patients were obtained from medical records. A standardized questionnaire including social-demographic characteristics was implemented in patients and control. Our study was approved by The Mercy Health Research Ethics Committee of Gansu Provincial Hospital (approval no. 003017). All patients provided written informed consent prior to enrollment in the study.

DNA Extraction and SNPs Genotyping

TIANamp Blood DNA Kit (DP348-03, Tiangen) was used to extract DNA from peripheral blood samples according to instructions. The genotyping of ERCC5 rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 was analyzed by MassARRAY iPLEX technology (Shanghai Genechem Co, Ltd). The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. The PCR reaction conditions were shown as follows: 95 °C for 2 minutes, 45 cycles at 95 °C for 30 seconds, annealing at 56 °C for 30 seconds, extension at 72 °C for 60 seconds, and final extension at 72 °C for 5 minutes. After desalted with resin, the Typer software automatically interprets the molecular weight peaks detected by the mass spectrometry, and the transformation shows the molecular mass spectrum peak map corresponding to the SNP site. The PCR primers are listed in Table 1.

Statistical Analysis

Statistical analysis was performed by using SPSS 17.0 software (SPSS Inc). The differences of social-demographic characteristics among patients in 3 groups were compared using $\chi^2$ test. The odds ratio (OR) values in cirrhosis group and liver cancer group were analyzed by regression analysis. $P$ value <.05 was considered as significant.

Results

Basic Information of the Control Group and Liver Cirrhosis, Liver Cancer Group

The study included 133 controls, 122 patients with cirrhosis and 110 patients with liver cancer. The number of male patients in the control group, the cirrhosis group, and liver cancer group were 80, 74, and 72, respectively, and the female patients were 53, 48, and 38. There was no significant difference in gender between the 3 groups ($P > .05$). In addition, the number of people drinking alcohol in the control group, the cirrhosis group, and liver cancer group were 80, 74, and 72, respectively, and the female patients were 53, 48, and 38. There was no significant difference in gender between the 3 groups ($P > .05$). In addition, the number of people drinking alcohol in the control group, the cirrhosis group, and liver cancer group were 53, 48, and 36, respectively. The number of people who did not drink alcohol was 76, 74, and 74. There were no significant differences in drinking between the 3 groups ($P > .05$). There were significant differences between the 3 groups in the age-group ≤60 and >60 subgroups, the patients in the liver cancer group were older
and the difference was significantly different ($P < .0001$; Table 2).

**Table 2. Basic Characteristics Among 3 Groups.**

| Characteristics | Control | Cirrhosis | Liver Cancer | $\chi^2$ | $P$ value |
|-----------------|---------|-----------|--------------|---------|-----------|
| Gender          |         |           |              |         |           |
| Male, n (%)     | 80 (35.4) | 74 (32.7) | 72 (31.9) | 0.8421 | .6563     |
| Female, n (%)   | 53 (38.1) | 48 (34.5) | 38 (27.4) |         |           |
| Drinking        |         |           |              |         |           |
| Yes, n (%)      | 57 (40.4) | 48 (34.1) | 36 (25.5) | 2.645  | .2664     |
| No, n (%)       | 76 (34.0) | 74 (33.0) | 74 (33.0) |         |           |
| Age (years)     | 65.3 ± 14.2 | 61.3 ± 16.4 |           | 0.037  |           |
| $\leq 60$, n (%) | 106 (48.0) | 74 (33.6) | 41 (18.4) | 45.37  | <.0001    |
| >60, n (%)      | 27 (18.8) | 48 (33.3) | 69 (47.9) |         |           |

**Table 3. Comparison of Genetic Loci Among Control Group, Cirrhosis Group, and Liver Cancer Group.**

| Loci          | Genotypes | Control | Cirrhosis | Liver Cancer | $\chi^2$ | $P$ value |
|---------------|-----------|---------|-----------|--------------|---------|-----------|
| rs2016073     |           |         |           |              |         |           |
| AA            | 67        | 72      | 54        | 27.577       | <.0001  |           |
| GG            | 20        | 18      | 0         |             |         |           |
| AG            | 46        | 32      | 56        |             |         |           |
| rs751402      |           |         |           |              |         |           |
| AA            | 20        | 26      | 0         | 39.497       | <.0001  |           |
| GG            | 67        | 72      | 54        |             |         |           |
| GA            | 48        | 24      | 56        |             |         |           |
| rs2094258     |           |         |           |              |         |           |
| CC            | 47        | 49      | 13        | 9.332        | .053    |           |
| TT            | 16        | 24      | 7         |             |         |           |
| CT            | 70        | 49      | 33        |             |         |           |
| rs2296148     |           |         |           |              |         |           |
| CC            | 128       | 122     | 101       | 9.636        | .008    |           |
| TC            | 7         | 0       | 9         |             |         |           |
| rs2296147     |           |         |           |              |         |           |
| CC            | 4         | 8       | 0         | 7.93         | .094    |           |
| TT            | 76        | 66      | 64        |             |         |           |
| CT            | 55        | 48      | 46        |             |         |           |
| rs2296148     |           |         |           |              |         |           |
| CC            | 128       | 122     | 101       | 9.636        | .008    |           |
| TC            | 7         | 0       | 9         |             |         |           |

Comparison of Genetic Loci Among Control Group, Cirrhosis Group, and Liver Cancer Group

In this study, ERCC5 rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 polymorphisms were analyzed. Our results found that locus rs2016073 was significant difference among 3 groups ($P < .0001$), and genotype GG was not observed in liver cancer group due to there was no genotype GG found in liver cancer group. As for locus rs751402, there were significant differences among 3 groups ($P < .0001$), and genotype AA was not observed in liver cancer group due to there was no genotype AA found in liver cancer group. As for locus rs2094258, there were significant differences among 3 groups ($P > .05$), but genotype CC was not observed in liver cancer group due to there was no genotype CC found in liver cancer group. For locus rs2296148, there were significant differences among 3 groups ($P < .05$), and genotype TC was not observed in cirrhosis group due to there was no genotype TC found in cirrhosis group (Table 3).

**Regression Analysis of Genetic Loci Among 3 Groups**

Regression analysis was performed for ERCC5 rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 polymorphisms between control group and cirrhosis group, as well as between control group and liver group. However, only locus rs751402 had significant difference between control group and cirrhosis group. The OR value of genotype AA in locus rs751402 was 2.600 (1.214-5.568), indicating that patients with genotype AA were more likely to have cirrhosis than those with genotype GA. And the OR value of genotype GG in locus rs751402 was 2.149 (1.189-3.886), indicating that patients with genotype GG were more likely to have cirrhosis than those with genotype GA (Table 4).

**Discussion**

As for DNA repair genes, there are many SNPs that may play an important role in impairing protein function and attenuating DNA repair capability, in which may cause genomic instability and individual predisposition to malignancies. Excision repair cross-complementing 5 can regulate DNA excision repair, and removal of bulky lesions caused by environmental chemicals or UV light. Excision repair cross-complementing 5 is a novel biomarker of ovarian cancer prognosis and a potential therapeutic target of ovarian cancer response to platinum chemotherapy. In this study, our results found that ERCC5 gene polymorphisms (rs2016073, rs751402, rs2094258, rs2296147, and rs2296148) were related to cirrhosis and liver cancer, indicating that ERCC5 gene polymorphisms may serve as new biomarkers for liver diseases.

Only one report found that ERCC5 promoter polymorphism (rs2016073) was shown a relationship with chemosensitivity of oxaliplatin-based chemotherapy in patients with advanced colorectal cancer. In our study, locus rs2016073 showed significant difference among 3 groups, genotypes AA and AG were found in 3 groups, but genotype GG was only found in liver cancer group, suggesting that genotype GG may be an import genotype to distinguish liver cancer from control and cirrhosis.

The association between SNPs in the ERCC5 promoter (rs751402) and development of gastric cancer in a Chinese population was found. Stratification by cancer type indicated that rs751402 polymorphism may increase the risk of gastric cancer and hepatocellular carcinoma, which was further confirmed by a false-positive report probability analysis. In addition, ERCC5 rs751402 polymorphism may be associated with risk of salivary gland tumors. In our study, locus rs751402 showed significant difference among 3 groups, and genotypes GG and GA were found in 3 groups, but genotype AA was only found in liver cancer group, suggesting that genotype AA may be an import genotype to distinguish liver cancer from control.
and cirrhosis. Patients with genotype AA or GG were more likely to have cirrhosis than those with genotype GA.

The rs2094258 polymorphism may be related to the increased risk of GC in Southern China.\(^ 18\) A case–control study found that \(ERCC5\) rs2094258 polymorphism may relate to the risk of breast cancer.\(^ {19}\) Previous study found that \(ERCC5\) rs2094258 showed no association with gastric cancer susceptibility.\(^ 8\) The same results were found in our study that rs2094258 was no significant difference among 3 groups, suggesting that rs2094258 was not associated with cirrhosis and liver cancer.

\(Excision\ \text{repair}\ \text{cross-complementing} 5\) variant rs2296147 T-allele creates a predicted TP53 binding site and upregulates transcript abundance in normal bronchial epithelial cells.\(^ {20}\) \(Excision\ \text{repair}\ \text{cross-complementing} 5\) rs2296147 reduced the risk of esophageal cancer, and the results of stratified analysis showed that rs2296147 could reduce the susceptibility to esophageal cancer in women, nonsmokers, drinkers, and nondrinkers.\(^ {21}\) \(Excision\ \text{repair}\ \text{cross-complementing} 5\) rs2296147 C variant genotypes were associated with a significantly lower ESCC risk.\(^ 6\) In our study, locus rs2296147 was no significant differences among 3 groups \((P > .05)\), indicating that locus rs2296147 may be not related to cirrhosis and liver cancer.

The last locus explored in our study was locus rs2296148. Only one study showed that 372C > T (rs2296148) was not associated with Clinical outcome of oxaliplatin-based chemotherapy in Chinese patients with advanced colorectal cancer.\(^ {22}\) As for locus rs2296148, there were significant differences among 3 groups \((P < .05)\), and genotype TC was not observed in cirrhosis group due to there was no genotype TC found in cirrhosis group. Our study provided a reference for rs2296148 to become a marker of liver cancer.

In conclusion, 5 \(ERCC5\) gene polymorphisms (rs2016073, rs751402, rs2094258, rs2296147, and rs2296148) were explored in our study, and our results found that loci (rs2016073, rs751402, and rs2296148) significant differences among control, cirrhosis, and liver cancer groups, especially genotype AA and genotype GG of rs751402 had significant higher OR value, indicating that they may be important targets for cirrhosis. \(Excision\ \text{repair}\ \text{cross-complementing} 5\) gene polymorphisms may exert important functions on cirrhosis and liver cancer. Due to small sample size and basic research, further validation by case–control studies with large samples is still needed.

**Authors' Note**

G.Y. and Y.Y. are cofirst authors. Our study was approved by The Mercy Health Research Ethics Committee of Gansu Provincial Hospital (approval no. 003017). All patients provided written informed consent prior to enrollment in the study.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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