RESEARCH ARTICLE

Polymorphisms of TERT and CLPTM1L and the Risk of Hepatocellular Carcinoma in Chinese Males

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Abstract

**Background:** Telomerase reverse transcriptase (TERT) and cleft lip and palate trans-membrane 1-like (CLPTM1L) genes located on chromosome 5p15.33 are known to influence the susceptibility to various cancers. Here, we examined the association of TERT and CLPTM1L single nucleotide polymorphisms (SNPs) with hepatocellular carcinoma (HCC). **Materials and Methods:** Genotyping of TERT SNP rs2736098 and CLPTM1L SNP rs401681 was performed using TaqMan allelic discrimination assays in a case-control study of 201 HCC cases and 211 controls in a Chinese male population. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression analyses. **Results:** Both the rs2736098 T allele of TERT and the rs401681 T allele of CLPTM1L were associated with a significantly increased risk of HCC (adjusted ORs=1.605, 95% CI=1.164-2.13; adjusted OR=1.399, 95% CI=1.002-1.955, respectively). **Conclusions:** Our results show that genetic variants of TERT and CLPTM1L may contribute to HCC susceptibility in Chinese males.

Keywords: Telomerase reverse transcriptase - cleft lip and palate transmembrane 1-like - SNPs - HCC risk

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Introduction

Primary liver cancer is the fifth most commonly diagnosed cancer worldwide but the second most frequent cause of death in men. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death. The highest reported primary liver cancer rates are found in East and South-East Asia. Hepatocellular carcinoma (HCC) represents the major histological subtype of primary liver cancers, accounting for 85-90% of the total liver cancer burden worldwide (Hashem et al., 2007; Global cancer statistics 2011). Meanwhile, liver cancer incidence rates are increasing in many parts of the world. The occurrence of HCC is a multi-factor and multi-stage process, including both hereditary and environmental factors. Long-term carcinogenic effects give rise to genetic changes, which can lead to tumor formation (Tanabe et al., 2008). In recent years, several studies have investigated the correlation of genetic polymorphisms and HCC.

The chromosome 5p15.33 region including telomerase reverse transcriptase (TERT) and cleft lip and palate trans-membrane 1-like (CLPTM1L) genes is known to be associated with the development of many cancers (Rafnar et al., 2009). Telomerase is a ribonucleoprotein complex composed of RNA and proteins that plays an important role in maintaining telomere stability, long-term cellular activity, and potential proliferation ability (Cong et al., 1999, Young 2010, Luis et al., 2011). Human TERT at the core of telomerase can synthesize DNA from an RNA template using catalytic activity. Recent studies have shown that the reactivation of TERT is likely to promote tumor progression and regulate cancer-promoting pathways (Artandi et al., 2010). CLPTM1L encodes a predicted transmembrane protein that was shown to induce apoptosis in cisplatin-resistant cell lines, although its role in tumor genesis remains unclear (Yamamoto et al., 2001).

To date, many studies have demonstrated that single nucleotide polymorphisms (SNPs) of TERT and CLPTM1L are associated with cancer risks. Two variants in this 5p15.33 region (rs401681 and rs2736098) are correlated with bladder cancer, lung cancer, glioma, breast cancer, and other tumors (Rafnar et al., 2009). However, the relationships between these SNPs and HCC have not been investigated. Therefore, we performed a case-control study consisting of 201 HCC cases and 211 controls to further research any correlation.
Materials and Methods

Our case-control study recruited 217 HCC patients aged 30-87 years from the first affiliated Hospital of China Medical University between 2008 and 2013. There were no restrictions on age or tumor stage. Controls were 210 non-cancer patients from the same hospital that were matched according to age (±5 years), gender (male), and ethnicity (Han Chinese). The diagnosis of HCC was confirmed by positive diagnostic imaging such as CT and angiography. The following HCC Characteristics were investigated from questionnaire interviews. A total of 201 cases of 217 selected HCC patients achieved a response rate of 93%. All participants were unrelated, ethnic Han Chinese individuals. Informed consent forms were obtained from all study individuals, and the China Medical University Ethics Committee approved this study.

DNA was extracted from peripheral blood using phenol-chloroform and ethanol precipitation as previously described (Sambrook 1989). rs2736098 and rs401681 genotyping was performed using Taqman allelic discrimination assays, and primers and probes were obtained from Applied Biosystems (Foster City, CA, USA). Polymerase chain reaction (PCR) conditions were 95°C for 10 min followed by 47 cycles of 92°C for 30 s and 60°C for 1 min. Results were read using Sequence Detection Software on an Applied Bio-systems 7500 FAST Real-Time PCR System according to the manufacturer’s instructions. For each SNP, genotypic frequencies were tested for deviation from Hardy-Weinberg Equilibrium using Pearson’s χ² test. There was no deviation in the control group. Distributions of demographic variables, risk factors, and genotypes between the cases and controls were evaluated using the two-sided χ² test. The associations of these two variant genotypes with risk of HCC were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses. Statistical analysis was carried out using two-sided tests with Statistical Product and Service Solutions software (v.16.0; SPSS Institute Cary, Chicago, IL, USA). p<0.05 was considered as the level of statistical significance.

Results

Patient and control characteristics are shown in Table 1. There were no significant differences in age distribution between the cases and controls (p=0.559), with a similar mean age in both groups (57.36±10.50 years for cases, and 58.04±12.18 years for controls). All individuals were male with different smoking statuses. However, the smoking status was not significantly different between cases and controls (p=0.229).

Both SNPs (TERT rs2736098 and CLPTM1L rs401681) were in Hardy-Weinberg Equilibrium (p=0.055 and 0.325, respectively). Detailed information about the genotype and allele distributions in cases and controls for the two SNPs is shown in Table 2. For the rs2736098 polymorphism, a significantly increased risk of HCC was associated with the CT genotype in a co-dominant model (CT vs CC: adjusted OR=1.840, 95%CI=1.148-2.948) and variant genotypes CT+TT in a dominant model (CT+TT vs CC: adjusted OR=1.946, 95%CI=1.244-3.043). We also found a significant association between the CLPTM1L rs401681 polymorphism and HCC risk for the CT genotype in a co-dominant model (CT vs CC:

| Table 1. Characteristics of HCC Cases and Controls |
|-----------------------------------------------|
| Characteristics | Cases (n=201) | Controls (n=211) | p values |
| Mean age | 57.36±10.50 | 58.04±12.21 | 0.522 |
| Gender(male) | 201 | 210 | |
| Smoker | 57(28.4%) | 71(34.1%) | 0.207 |
| Non-smoker | 84(41.8%) | 139(65.9%) | |
| HBV(Positive) | 161(80.1%) | 170(80.7%) | |
| HBV(Negative) | 40(19.9%) | 37(19.3%) | |
| AFP <20 | 74(36.8%) | 103(48.9%) | |
| AFP>20 | 107(53.2%) | 98(46.1%) | |
| TNM=I+II | 93(46.3%) | 80(37.9%) | |
| TNM III+IV | 108(53.7%) | 131(62.1%) | |
| lymph nod metastasis (N) | 173(86.1%) | 195(92.3%) | |
| lymph nod metastasis (Y) | 37(19.9%) | 36(17.1%) | |
| Tumor number=1 | 104(51.7%) | 91(43.7%) | |
| Tumor number=2 | 97(48.3%) | 120(56.3%) | |

Two-sided test

| Table 2. TERT (rs2736098) and CLPTM1L (rs401681) Genotype Frequencies in HCC Cases and Controls and their Associations with HCC Risk |
|-----------------------------------------------|
| rs2736098 | Cases (N) | Controls (N) | p values | Adjusted OR (95%CI) |
| CC | 75(37.3) | 111(52.8) | 1 |
| CT | 97(48.3) | 76(36.2) | 0.011 | 1.840(1.148-2.948) |
| TT | 29(14.4) | 23(11.0) | 0.018 | 2.406(1.160-4.899) |
| CT+TT | 126(62.7) | 99(47.2) | 0.004 | 1.946(1.244-3.043) |
| T allele | 0.004 | 1.605(1.164-2.213) |
| rs401681 | |
| CC | 86(42.8) | 120(57.2) | 1 |
| CT | 98(48.7) | 75(35.2) | 0.016 | 1.786(1.115-2.863) |
| TT | 18(8.5) | 16(7.6) | 0.333 | 1.520(0.651-3.546) |
| CT+TT | 115(57.2) | 90(42.7) | 0.016 | 1.739(1.110-2.723) |
| T allele | 0.049 | 1.399(1.002-1.955) |

*Adjusted by age

| Table 3. Association between Joint Analysis of rs2736098 and rs401681 and HCC Risk |
|-----------------------------------------------|
| TERT | CLPTM1L | Cases (N) | Controls (N) | P values | Adjusted OR (95%CI) |
| CC | CC | 25 | 51 | 0.086 | 1.700(0.925-3.123) |
| CT+TT | CC | 61 | 69 | 0.049 | 1.803(1.000-3.252) |
| CT+TT | CT+TT | 65 | 30 | 0.000 | 4.420(2.319-8.425) |

**Adjusted by age

| Table 4. Interaction Analysis between Polymorphisms and Smoking with HCC Risk |
|-----------------------------------------------|
| Genotypes | Cases (N) | Controls (N) | P values | Adjusted OR (95%CI) |
| Smoking status TERT rs2736098 |
| Non-smokers | CC | 35 | 68 | 1 |
| Non-smokers | CT+TT | 49 | 71 | 0.55 | 1.211(0.647-2.269) |
| Smokers | CC | 23 | 43 | 0.754 | 0.888(0.422-1.868) |
| Smokers | CT+TT | 34 | 28 | 0.022 | 2.353(1.133-4.887) |
| CLPTM1L rs401681 |
| Non-smokers | CC | 37 | 75 | 1 |
| Non-smokers | CT+TT | 47 | 64 | 0.177 | 1.545(0.822-2.904) |
| Smokers | CC | 26 | 45 | 0.891 | 1.051(0.518-2.130) |
| Smokers | CT+TT | 31 | 26 | 0.009 | 2.790(1.296-6.008) |

*Adjusted by age
adjusted OR=1.786, 95% CI=1.115-2.863) and variant genotypes CT+TT in a dominant model (CT+TT vs CC: adjusted OR=1.739, 95% CI=1.110-2.723).

Table 3 shows the association between the joint effect of TERT and CLPTM1L genotypes and HCC risk. We used the TERT CC genotype and CLPTM1L CC genotype as references to determine high-risk genotypes and found that CT+TT of TERT and CT+TT of CLPTM1L had a significantly increased risk of HCC with an adjusted OR of 4.420 (95% CI= 2.319-8.425, p≤0.001). The results of the analysis into the interaction between TERT rs2736098, CLPTM1L rs401681, and smoking with the risk of HCC are shown in Table 4. Smokers carrying both TERT and CLPTM1L risk genotypes had a higher risk of HCC (adjusted OR=2.353, 95% CI=1.133-4.887; adjusted OR=2.790, 95% CI=1.296-6.008).

The association of the two SNPs with clinical indicators hepatitis B virus (HBV) and alpha-fetoprotein (AFP) was next analyzed in HCC patients (Tables 5 and 6). There was no significant association in the genotype frequency between HBV and non-HBV HCC patients, but the TERT rs2736098 T allele was associated with an increased level of AFP (co-dominant model CC vs TT: OR=3.354, 95% CI=1.226-9.177; dominant model: OR=1.952,

Table 5. Association of rs2736098 Genotypes with Relative Factors in HCC Patients

| Genotype | Cases (N) | OR (95%CI) | P   | OR (95%CI) | P   | OR (95%CI) | P   |
|----------|-----------|------------|-----|------------|-----|------------|-----|
| rs2736098 |           |            |     |            |     |            |     |
| CC       | 18/57     | 1.493(0.740-3.011) | 0.263 | 1.226(0.437-3.444) | 0.699 |
| CT       | 17/80     | 1.486(0.706-3.130) | 0.297 |
| TT       | 5/24      | 1.516(0.505-4.552) | 0.458 |

Table 6. Association of rs401681 Genotypes with Relative Factors in HCC Patients

| Genotype | Cases (N) | OR (95%CI) | P   | OR (95%CI) | P   | OR (95%CI) | P   |
|----------|-----------|------------|-----|------------|-----|------------|-----|
| rs401681 |           |            |     |            |     |            |     |
| CC       | 18/68     | 1.119(0.557-2.247) | 0.752 | 4.303(0.553-33.462) | 0.163 |
| CT       | 21/77     | 0.971(0.487-1.972) | 0.934 |
| TT       | 16-Jan    | 4.235(0.526-34.106) | 0.175 |

Table 7. Association between TERT rs2736098 and Cancer Progress

| Genotype | Cases (N) | OR (95%CI) | P   | OR (95%CI) | P   |
|----------|-----------|------------|-----|------------|-----|
| rs2736098 |           |            |     |            |     |
| CC       | 30/45     | 0.667(0.374-1.190) | 0.170 | 1.070(0.485-2.360) | 0.866 |
| CT       | 50/47     | 0.627(0.341-1.153) | 0.133 |
| TT       | 13/16     | 0.821(0.345-1.950) | 0.654 |

Table 8. Association between CLPTM1L rs401681 and Cancer Progress

| Genotype | Cases (N) | OR (95%CI) | P   | OR (95%CI) | P   |
|----------|-----------|------------|-----|------------|-----|
| rs401681 |           |            |     |            |     |
| CC       | 38/48     | 0.667(0.374-1.190) | 0.170 | 1.070(0.485-2.360) | 0.866 |
| CT       | 49/49     | 0.792(0.443-1.416) | 0.431 |
| TT       | 11-Jun    | 1.451(0.492-4.282) | 0.500 |

\*TNM; \*No lymph node metastasis; \*Lymph node metastasis; \*The number of tumors.
TERT induces genotoxic stress and DNA damage, which would contribute to HCC in male individuals. The dominant model also indicated that any CT genotype increased the HCC risk in a Chinese male population. The interaction between the two SNPs and cancer progress.

Limited knowledge is available regarding the function of CLPTM1L, although it is thought to contribute to the accumulation of DNA damage and cell apoptosis (Myneni et al., 2013). CLPTM1L over-expression is also reported in many human cancers, such as lung cancer, skin cancer, and glioma (Harley et al., 1990; Iwama et al., 1998; Rudolph et al., 2001). The TERT gene spans 35 kb of genomic DNA, and consists of 16 exons and 15 introns. The rs2736098 polymorphism is located within the second exon (Wick et al., 1999); however, its function is unclear and its effect on the molecular mechanism of HCC risk has not been ascertained. Our data provide strong evidence from a Chinese population that the rs2736098 [T] polymorphism increases susceptibility to HCC, which supports a previous study carried out in Tianjin (Zhang et al., 2012).

Limited knowledge is available regarding the function of CLPTM1L, although it is thought to contribute to the accumulation of DNA damage and cell apoptosis (Myneni et al., 2013). CLPTM1L over-expression is also reported in many human cancers, such as lung cancer (Wang et al., 2008; Ni et al., 2012). However, study findings are controversial. According to HapMap data, these discrepancies could reflect different allele frequencies in different ethnicities, or might be affected by cancer types, hereditary factors, and environmental factors (Choi et al., 2009). No report has previously determined whether the CLPTM1L rs401681 polymorphism influences HCC susceptibility, but we found that the CLPTM1L rs401681 CT genotype increased the HCC risk in a Chinese population. The dominant model also indicated that any T (CT+TT) genotypes of rs401681 were associated with HCC in male individuals.

Our analysis found that carrying the TERT rs2736098 T allele and CLPTM1L rs401681 T allele increased the risk of developing HCC. One explanation for this derives from a previous study that speculated that CLPTM1L might be in strong linkage disequilibrium with other potential functional SNPs (Yamamoto et al., 2001; Zhao et al., 2012). In addition, CLPTM1L was reported to be involved in cellular responses to genotoxic stress and cisplatin resistance (James et al., 2012). Many factors can induce genotoxic stress and DNA damage, which would lead to the malignant transformation of cells. Smoking is considered to be a major factor (Myneni et al., 2013), and, indeed, our data showed that individuals carrying TERT rs2736098 or CLPTM1L rs401681 risk genotypes and who smoked had a significant association with HCC (p=0.022 and 0.009, for respective heterogeneity).

The association of the two SNPs with clinical indicators HBV and AFP and HCC progress was analyzed in HCC patients, but only a significant association between the TERT T allele and AFP was identified. The rearrangement and instability of genomic DNA could occur through the integration of HBV DNA and hepatocyte chromosomal DNA (Zhang et al., 2012). Indeed, recent reports showed that the telomerase gene was targeted for integration in independent HCCs and HCC-derived cell lines (Brechot, 2004). However, we did not obtain significant results regarding HBV and SNP associations. This discrepancy could reflect the analysis of only the male gender in the present study and its small sample size. AFP is regarded as the most useful serum biomarker for HCC patients, with a critical value of 20 serum proteins being commonly used in the early diagnosis of HCC (Shen et al., 2012). Our results showed that patients carrying the rs2736098 T allele were associated with a significantly increased AFP level.

To our knowledge, this is the first study to investigate the effects of TERT rs2736098 and CLPTM1L rs401681 SNPs in a Chinese Han population on HCC susceptibility. Although our study was limited regarding its small sample size and lack of associated risk factors such as alcohol use, we nevertheless identified prominent associations with HCC and TERT and CLPTM1L polymorphisms, gene-gene interactions, and gene-risk factor interaction. The TERT rs2736098 T allele and CLPTM1L rs401681 T allele increased the risk of HCC, and the association of TERT and CLPTM1L with HCC was stronger in smokers. The TERT rs2736098 T allele was also associated with increased AFP levels. Neither of the two SNPs had an association with HCC progress. Further work will be necessary to confirm these findings and to investigate more HCC risk factors in larger sample sizes and in different ethnicities.

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Conceived and designed the experiments: LYS. Performed the experiments: LYS XLL LS YZ MMZ. Analyzed the data: LYS LS YZ. Contributed reagents/materials/analysis tools: LYS ZHY HYS BSZ. Wrote the paper: LYS.

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