Introduction

Hepatoblastoma is the most common hepatic tumor of childhood (1,2). The incidence of hepatoblastoma is about 0.5–1.5 cases per million, and the mortality rate can be as high as 35–50% for high-risk patients (3). Over the past decades, efforts have been made to improve the outcome of hepatoblastoma. However, treatment has not changed significantly in the past 20 years (4). In recent years, several unique genetic features have been identified to be associated with hepatoblastoma, providing new insights into the understanding of hepatoblastoma (5). The elucidation of the genetic features of hepatoblastoma is thus of critical importance.

Single-nucleotide polymorphisms (SNPs) are the most common sources of genetic variation in the genome and are frequently associated with potential cancer risk (6). Some SNPs contributing to the progression of hepatoblastoma have been identified. Arai et al. revealed that MDM4 polymorphisms are significantly correlated with the outcomes of hepatoblastoma (7). Based on high-density SNP genotyping microarrays, Suzuki et al. demonstrated...
that expression levels of \textit{IGF2} and \textit{H19} were significantly correlated with hepatoblastoma (8). \textit{c-Myc} is a well-known human transcription factor involved in cell cycle, growth, metabolism, and apoptosis (9). A previous study showed that the \textit{CMYC} rs6883267 polymorphism is significantly associated with \textit{CMYC} transcription efficiency and poor prognosis in colorectal cancer (10). However, the association between \textit{CMYC} polymorphisms and hepatoblastoma remains unclear. This study therefore aimed to investigate the association of \textit{CMYC} polymorphisms with hepatoblastoma susceptibility.

\section*{Methods}

\subsection*{Patients}

Patients less than 18 years old with a pathologic diagnosis of hepatoblastoma were enrolled. Cancer-free control subjects matched for age and sex were recruited from the same area. All patients and control subjects were genetically unrelated members of the Chinese Han population. Written informed consent was acquired from all participants’ legal guardians or parents. The institutional review board of Guangzhou Women and Children’s Medical Center approved this study. All patient data were anonymous or de-identified prior to analysis.

\subsection*{CMYC genotyping}

Allelic discrimination of the rs4645943 and rs2070583 polymorphisms of \textit{CMYC} was performed using TaqMan reagents (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol, as reported previously (11-14). Control samples of known genotype were also included in each test, including blank, homozygous wild-type, homozygous mutant, and heterozygous samples. Quality control was performed with eight negative and positive control samples on each of the 384-well plates; 10% of the samples were also randomly selected for a second round of genotyping, and the concordance rate was 100%.

\subsection*{Statistical analysis}

All statistical analyses were performed with SAS software (version 9.1; SAS Institute, Cary, NC, USA). Continuous variables were analyzed using Student’s \textit{t}-test or one-way analysis of variance. Categorical variables were analyzed by \textit{\chi}^2 test. Differences in allele or genotype frequencies between patients and controls were determined by \textit{\chi}^2 test. Hardy-Weinberg equilibrium (HWE) was calculated using a goodness-of-fit \textit{\chi}^2 test for biallelic markers. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for evaluation of the strength of the association of interest (15-17). Adjusted ORs were calculated using multivariate analysis after adjusting for age, sex, and clinical stage. Differences were considered significant at \textit{P}<0.05.

\section*{Results}

\subsection*{Characteristics of participants enrolled in this study}

A total of 213 hepatoblastoma patients and 958 control subjects were recruited from Guangdong, Henan, Shanxi, and Shanxi provinces in China. Males made up the majority of both the hepatoblastoma and control groups, accounting for 60.56% and 60.44% of individuals, respectively. Most of the patients had stage II disease (n=55), followed by stage I (n=42), stage III (n=40), and stage IV (n=15); stage information was lacking for 61 patients (Table S1). There were no significant differences between cases and controls regarding the distributions of age and sex (\textit{P}>0.05, Table 1).

\subsection*{Association between CMYC polymorphisms and hepatoblastoma risk}

Genotype distributions and associations between \textit{CMYC} gene polymorphisms and hepatoblastoma risk are summarized in Table 2. For rs4645943, compared with carriers of the CC genotype, carriers of the CT (OR, 1.10; 95\% CI, 0.81-1.51; \textit{P}=0.532) or TT (OR, 1.10; 95\% CI, 0.63-1.92; \textit{P}=0.726) genotypes showed no significant associations with hepatoblastoma risk. Moreover, there was no significant association between rs4645943 and hepatoblastoma risk under the additive (OR, 1.07; 95\% CI, 0.85-1.35; \textit{P}=0.550), dominant (OR, 1.10; 95\% CI, 0.82-1.49; \textit{P}=0.512), or recessive models (OR, 1.06; 95\% CI, 0.62-1.81; \textit{P}=0.842). For rs2070583, compared with carriers of the AA genotype, carriers of the AG (OR, 1.12; 95\% CI, 0.80-1.55; \textit{P}=0.516) and GG (OR, 0.84; 95\% CI, 0.35-2.04; \textit{P}=0.699) genotypes exhibited no significant associations with hepatoblastoma risk. Similarly, there was no significant association between rs2070583 and hepatoblastoma risk under the additive (OR, 1.04; 95\% CI, 0.79-1.36; \textit{P}=0.783),
dominant (OR, 1.08; 95% CI, 0.79–1.49; P=0.618), or recessive models (OR, 0.81; 95% CI, 0.34–1.97; P=0.645).

In addition, we found no significant association between hepatoblastoma risk and the combination of the rs4645943 CT/TT genotype with the rs2070583 AA/AG genotype (OR, 1.13; 95% CI, 0.84–1.53; P=0.410).

Stratification analysis of CMYC genotypes and hepatoblastoma risk

Further analysis showed that neither CMYC polymorphism was significantly associated with hepatoblastoma risk in any of the subgroups of hepatoblastoma patients (Table 3), which were stratified according to age, sex, and clinical tumor stage (all P>0.05). In addition, the combination of the rs4645943 CT/TT and rs2070583 AA/AG genotypes was not significantly associated with hepatoblastoma risk in any subgroups stratified by age, sex, or clinical tumor stage (all P>0.05). These findings suggest that CMYC polymorphisms are not significantly associated with hepatoblastoma susceptibility.

Discussion

Our results showed that the CMYC rs4645943 and rs2070583 polymorphisms were not associated with hepatoblastoma susceptibility. Further stratification analysis based on age, sex, and clinical stage found similar results.

CMYC, encoding the c-Myc protein, is an important oncogene involved in many steps of tumorigenesis, such as proliferation, survival, apoptosis, migration, and invasion (18). A previous study revealed that the expression of c-Myc and cyclin-D1 was significantly elevated in pretreated hepatoblastoma samples but decreased after chemotherapy (19). Myc-expressing mice can present with hepatocellular carcinoma and hepatoblastoma-like tumors, but tumor regression can be induced by inhibiting the expression of Myc (20). Hartwell et al. demonstrated that prolactin suppresses hepatocellular carcinoma by inhibiting the innate immune activation of c-Myc in a mouse model (21). Han et al. found that miR-148a-5p and miR-363-3p negatively regulate the expression of c-Myc to modulate hepatocarcinogenesis (22). These findings suggest that the abnormal expression of CMYC may play a critical role in the development of liver cancer.

SNPs may be associated with gene transcriptional activity (20,23). For example, CMYC polymorphisms are cis-regulated in the immortalized lymphocytes of HapMap individuals (23). Lee et al. revealed that the CMYC rs4645943 polymorphism was associated with the

| Variables | Cases (n=213), N (%) | Controls (n=958), N (%) | P |
|-----------|---------------------|------------------------|---|
| Age range, months | 0.23–149.97 | 0.004–156.000 | 0.105 |
| Mean ± SD | 23.62±24.36 | 23.75±18.30 | |
| <17 | 114 (53.52) | 454 (47.39) | |
| ≥17 | 99 (46.48) | 504 (52.61) | |
| Sex | | | 0.973 |
| Female | 84 (39.44) | 379 (39.56) | |
| Male | 129 (60.56) | 579 (60.44) | |
| Clinical stages | | | – |
| I | 42 (19.72) | – | |
| II | 55 (25.82) | – | |
| III | 40 (18.78) | – | |
| IV | 15 (7.04) | – | |
| NA | 61 (28.64) | – | |

1, Two-sided χ² test for distributions between hepatoblastoma patients and cancer-free controls; †, stage information was absent. SD, standard deviation; NA, not applicable.
| Genotype       | Cases (N=213) | Controls (N=958) | P†  | Crude OR (95% CI) | P   | AOR (95% CI)‡ | P‡  |
|----------------|---------------|------------------|-----|------------------|-----|----------------|-----|
| rs4645943 C>T   |               |                  |     |                  |     |                |     |
| CC             | 105 (49.30)   | 496 (51.77)      | 1.00| 1.00             | 1.00|                |     |
| CT             | 90 (42.25)    | 385 (40.19)      |     | 1.10 (0.81–1.51) | 0.533| 1.10 (0.81–1.51) | 0.532|
| TT             | 18 (8.45)     | 77 (8.04)        |     | 1.10 (0.63–1.92) | 0.726| 1.10 (0.63–1.92) | 0.726|
| Additive       | –             | –                | 0.807| 1.07 (0.85–1.35) | 0.550| 1.07 (0.85–1.35) | 0.550|
| Dominant       | 108 (50.70)   | 462 (48.23)      | 0.513| 1.10 (0.82–1.49) | 0.513| 1.10 (0.82–1.49) | 0.512|
| Recessive      | 195 (91.55)   | 881 (91.96)      | 0.842| 1.06 (0.62–1.81) | 0.842| 1.06 (0.62–1.81) | 0.842|
| rs2070583 A>G   |               |                  |     |                  |     |                |     |
| AA             | 143 (67.14)   | 660 (68.89)      | 1.00| 1.00             | 1.00|                |     |
| AG             | 64 (30.05)    | 265 (27.66)      |     | 1.12 (0.80–1.55) | 0.516| 1.12 (0.80–1.55) | 0.516|
| GG             | 6 (2.82)      | 33 (3.44)        |     | 0.84 (0.35–2.04) | 0.699| 0.84 (0.35–2.04) | 0.699|
| Additive       | –             | –                | 0.727| 1.04 (0.79–1.36) | 0.783| 1.04 (0.79–1.36) | 0.783|
| Dominant       | 70 (32.86)    | 298 (31.11)      | 0.617| 1.08 (0.79–1.49) | 0.617| 1.08 (0.79–1.49) | 0.618|
| Recessive      | 207 (97.18)   | 925 (96.56)      | 0.644| 0.81 (0.34–1.96) | 0.645| 0.81 (0.34–1.97) | 0.645|
| Combined effect of risk genotypes§ |               |                  |     |                  |     |                |     |
| 0–1            | 111 (52.11)   | 529 (55.22)      | 1.00| 1.00             | 1.00|                |     |
| 2              | 102 (47.89)   | 429 (44.78)      | 0.410| 1.13 (0.84–1.53) | 0.410| 1.13 (0.84–1.53) | 0.410|

†, χ² test for genotype distributions between hepatoblastoma patients and cancer-free controls; ‡, adjusted for age and sex; §, risk genotypes were carriers with rs4645943 CT/TT and rs2070583 AA/AG genotypes. AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.
Table 3 Stratification analysis for association between CMYC genotypes and hepatoblastoma susceptibility

| Variables   | rs4645943 (case/control) | AOR (95% CI)† | P† | rs2070583 (case/control) | AOR (95% CI)† | P† | Combine genotypes (case/control) | AOR (95% CI)† | P† |
|-------------|---------------------------|---------------|----|--------------------------|---------------|----|----------------------------------|---------------|----|
|             | CC                        | CT/TT         |     | AA                       | AG/GG         |     | 0–1                              |               |    |
| Age, months |                           |               |     |                          |               |     |                                  |               |    |
| <17         | 53/235                    | 61/219        |     | 1.24 (0.82–1.86)         | 0.315         |     | 56/250                           | 58/204        | 1.27 (0.84–1.91) 0.257 |
| ≥17         | 52/261                    | 47/243        |     | 0.97 (0.63–1.49)         | 0.892         |     | 55/279                           | 44/225        | 0.99 (0.64–1.53) 0.971 |
| Sex         |                           |               |     |                          |               |     |                                  |               |    |
| Female      | 38/196                    | 46/183        |     | 1.31 (0.81–2.10)         | 0.273         |     | 42/211                           | 42/168        | 1.27 (0.79–2.03) 0.332 |
| Male        | 67/300                    | 62/279        |     | 1.00 (0.68–1.46)         | 0.986         |     | 69/318                           | 60/261        | 1.06 (0.72–1.56) 0.763 |
| Clinical stage |                         |               |     |                          |               |     |                                  |               |    |
| I + II      | 43/496                    | 54/462        |     | 1.35 (0.88–2.05)         | 0.166         |     | 48/529                           | 49/429        | 1.26 (0.83–1.91) 0.285 |
| III + IV    | 27/496                    | 28/482        |     | 1.11 (0.65–1.91)         | 0.703         |     | 28/529                           | 27/429        | 1.19 (0.69–2.05) 0.536 |

†, Adjusted for age and sex, omitting the corresponding stratification factor. AOR, adjusted odds ratio; CI, confidence interval.
warfarin dose requirement in patients undergoing cardiac valve replacement (24). Moreover, the CMYC rs2070583 polymorphism is significantly associated with coronary heart disease in African Americans (25). However, in the current study, no significant associations were found between the CMYC rs4645943 and rs2070583 polymorphisms and hepatoblastoma susceptibility in a Han Chinese population. Therefore, we speculate that abnormal expression of CMYC in hepatoblastoma may not be attributed to CMYC gene polymorphisms. Wang et al. demonstrated that the role of CMYC in hepatoblastoma is to impose mutually dependent alterations in gene expression and metabolic re-programming that are not obtained in non-transformed cells and that cooperate to promote tumor growth (26). The activation of β-catenin is one of the hallmarks of hepatoblastoma, inducing its translocation to the nucleus and activating target genes, including CMYC, MMP genes, and VEGF to regulate cell proliferation, invasion, and angiogenesis (27). As a member of the Wnt signaling pathway, Wnt ligand binding suppresses the phosphorylation of β-catenin to inhibit its downstream target genes, such as CMYC, repressing cell proliferation (28). Taken together, this evidence suggests that the abnormal expression of CMYC (or the protein c-Myc) in hepatoblastoma may largely depend on the regulation of upstream effectors, rather than its genetically encoded information.

Some limitations of this study should be mentioned. First, although we tried to recruit a large number of hepatoblastoma patients, the sample size in this study was still relatively small, and more patients are required to further validate our findings. Second, due to a lack of detailed information on the patients, associations between CMYC polymorphisms and clinical characteristics, such as tumor size and lymph node metastasis, were not analyzed in this study. Lastly, the study population does not represent the complete Chinese population.

Conclusions

In summary, the CMYC rs4645943 and rs2070583 polymorphisms may not be associated with hepatoblastoma risk. The abnormal regulation of CMYC in hepatoblastoma may therefore require further investigations and explanation.

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Footnote

Conflicts of Interest: The authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.12.19). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was acquired from all participants’ legal guardians or parents. The institutional review board of Guangzhou Women and Children’s Medical Center approved this study (No. 2017120101). All patient data were anonymous or de-identified prior to analysis.

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| Variables          | Guangdong province | Henan province | Shaanxi province | Shanxi province |
|--------------------|--------------------|----------------|------------------|----------------|
|                    | Cases (n=146), Controls (n=438), n (%) | Cases (n=42), Controls (n=176), n (%) | Cases (n=15), Controls (n=186), n (%) | Cases (n=10), Controls (n=158), n (%) |
| Age range, months  | 0.63–149.97        | 0.07–156.00    | 0.83–108.00      | 0.03–60.00     |
|                    | 0.214              | 0.285          | 0.286            | 0.785          |
| Mean ± SD          | 23.16±24.59        | 23.11±18.62    | 26.73±24.96      | 23.66±16.66    |
|                    | 0.285              | 0.285          | 0.285            | 0.285          |
| <17                | 79 (54.11)         | 211 (48.17)    | 21 (50.00)       | 65 (40.00)     |
|                    | 0.961              | 0.830          | 0.544            | 0.912          |
| ≥17                | 67 (45.89)         | 227 (51.83)    | 21 (50.00)       | 52 (60.00)     |
|                     | 0.961              | 0.830          | 0.544            | 0.912          |
| Gender             | Female             | Male           | Female           | Male           |
|                    | 58 (39.73)         | 88 (60.27)     | 15 (35.71)       | 27 (64.29)     |
|                    | 175 (39.95)        | 263 (60.05)    | 66 (37.50)       | 110 (62.50)    |
|                    | 0.961              | 0.830          | 0.544            | 0.912          |
| Clinical stages    | I                  | II             | III               | IV             |
|                    | 6 (4.11)           | 46 (31.51)     | 37 (25.34)       | 12 (8.22)      |
|                    | –                  | 3 (7.14)       | 3 (7.14)         | 1 (2.38)       |
|                    | 0.285              | 0.285          | 0.285            | 0.285          |
|                    | 19 (45.24)         | 3 (7.14)       | 3 (7.14)         | 1 (2.38)       |
|                    | –                  | –              | –                | –              |
|                    | 15 (100.00)        | –              | –                | –              |
|                    | 15 (100.00)        | –              | –                | –              |
|                    | –                  | –              | –                | –              |
|                    | 2 (20.00)          | –              | –                | –              |
|                    | 6 (60.00)          | –              | –                | –              |
|                    | 6 (60.00)          | –              | –                | –              |
|                    | 4 (40.00)          | –              | –                | –              |
|                    | 66 (41.77)         | –              | –                | –              |
|                    | 92 (58.23)         | –              | –                | –              |

1, Two-sided \(^2\) test for distributions between hepatoblastoma patients and cancer-free controls. SD, standard deviation; NA, not applicable.