Von Hippel-Lindau gene single nucleotide polymorphism (rs1642742) may be related to the occurrence and metastasis of HBV-related hepatocellular carcinoma

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Abstract

It is well-known that microRNAs are able to regulate the expression of target mRNAs through complementary base-pairing to their 3' untranslated regions (3'UTR) sequences. This study aimed to investigate whether single nucleotide polymorphisms resided in the 3'UTR sequences of several genes regulated by microRNAs were related to the occurrence and metastasis of hepatocellular carcinoma (HCC). Seventeen single nucleotide polymorphisms in the 3'UTR sequence of 10 genes regulated by miRNA were identified by bioinformatics methods and were 2,004 patients with HBV-related HCC and 216 patients with chronic HBV infection were recruited. Through case-control study, only found that the von Hippel-Lindau gene rs1642742 (G>A) may be associated with the occurrence and metastasis of HCC. The ORs of the frequencies of rs1642742 A allele versus G allele were 1.424 (95% confidence interval CI=1.019–1.989) between HBV-related HCC and chronic HBV infection group and were 2.004 (95% CI=1.031–3.895) between tumor metastasis and non-metastasis group, respectively. Through multivariate regression analysis, we also found that rs1642742 AA genotype was an independent risk factor for tumor metastasis (odds ratio =2.227, 95% CI =1.043–4.752, P =0.038) in HBV-related HCC group. Our study suggested that Von Hippel-Lindau rs1642742 contributed to susceptibility to developing HCC and correlated with tumor metastasis.

Keywords: hepatitis B virus, hepatocellular carcinoma, miRNA, VHL, microRNA, single nucleotide polymorphism

1. Introduction

Hepatitis B virus-related hepatocellular carcinoma (HBV-related HCC), which is caused by chronic infection of HBV, is one of the most common cancers and a serious threat to public health worldwide. Although chronic HBV infection causes more than 50% of HCC cases worldwide and is the dominant risk factor for HCC in China, only about 14.2% of patients chronically infected with HBV suffer from HCC in their lifetime. This indicates that the occurrence of HBV-related HCC is affected by several factors including both viral and host factor and then many studies have been conducted to screen out risk factors for HBV-related HCC. Until recently, viral factors, including HBV genotypes and mutations, have been identified to affect the risk of HBV-related HCC. Hepatitis B virus X protein (HBx protein), another viral factor, is found out to play a vital role in the development of HBV-related HCC. Mounting evidences reveal that HBs is able induce HCC through activating NF-kappa B signaling pathway, JAK-STAT signaling pathway, PI3K-Akt signaling pathway, and MAPK signaling pathway. These signaling pathway genes including Transcription factor p65, Proto-oncogene c-Rel, NFKB inhibitor alpha, Janus kinase, Signal transducer and activator of transcription, Protein inhibitor of activated STAT, Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit, Protein kinase, Matrix metallopeptidase, and so on. More than that, HBx could also promote carcinogenesis through activating the expression of many important genes including TNF-α, CA9, c-Jun, and so on.

Meanwhile, growing evidences demonstrate that host genetic background also contributes to the risk of HBV-related HCC. MicroRNAs (miRNAs) are 18 to 25 nucleotides (nt), single-stranded non-coding small RNAs. It is well-known that miRNAs are able to regulate the expression of target mRNAs through complementary base-pairing to their 3'-untranslated regions.
regions (3'UTR) sequences. \cite{12} Single nucleotide polymorphisms (SNPs) are the most abundant genetic markers in human genome. \cite{13} Because sequence complementarity and binding thermodynamics is important for the interaction between miRNAs and their targets, \cite{14} SNPs in the miRNA-binding sites may disrupt the interaction between miRNAs and their target mRNAs, modulate the expression of these genes and influence the risk of several diseases including non-small cell lung cancer, \cite{15} autism, \cite{16} breast cancer, \cite{17} Burkitt lymphoma, \cite{18} esophageal squamous cell carcinoma, \cite{19} and so on. Recent experimental evidences show that several SNPs in the miRNA binding sites, such as rs1292037, \cite{20} rs2596542, \cite{21} rs1057317, \cite{22} and rs7963551, \cite{23} elevate the risk of HCC. Because of the importance of above-mentioned signaling pathways genes regulated by HBx, we speculate that SNPs in miRNA-binding sites, which binding in the 3'UTR sequences of these genes, may lead to aberrant gene expression and influence the risk of occurrence HBV-related HCC. To date, there are few studies that have investigated the association between the SNPs in miRNA-binding sites binding with the genes regulated or affected by HBx and HBV-related HCC. In this study, we tried to investigate whether these SNPs could increase or decrease the risk of occurrence HBV-related HCC. With the use of bioinformatic tools, the putative functional SNPs in the miRNAs binding sites were identified and selected for this study. Through case-control studies, we found that SNP in miRNA-binding sites maybe related to the occurrence and metastasis of HBV-related HCC for the first time. This study provides new insights into the developing and progression of HBV-related HCC in viral and host factors.

2. Methods and materials

2.1. Ethics approval

All clinical work was conducted in accordance with the ethical regulations of World Medical Association Declaration of Helsinki (1996) after the approval of the ethical committee of Faculty of the People’s Hospital of Deyang City, Sichuan Province, China. All methods were performed in accordance with the relevant guidelines and regulations. Patients or legal guardians received detailed information about the study, and they signed an informed consent form.

2.2. Study population

Between September 2016 and December 2018, we consecutively enrolled HBV-related HCC patients who were admitted to People’s Hospital of Deyang City. Patients with chronic HBV infection who matched the gender and age of HBV-related HCC patients were also recruited. The detailed inclusion and exclusion criteria in the current study were as follows: clinical diagnosed HCC patients; hepatitis B surface antigen positive for more than 6 months; negative test results for hepatitis A, C, D, and E viruses, cytomegalovirus, Epstein-Barr virus, and HIV; no decompensated cirrhosis, liver failure; and no alcoholic hepatitis, autoimmune hepatitis, and fatty liver. All subjects were ethnic Han Chinese. HCC and chronic HBV infection were diagnosed according to “Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases” \cite{24} and “The guideline of prevention and treatment for chronic hepatitis B: a 2015 update.” \cite{25} Informed consents were obtained from all the patients involved in this study. Clinical information (including HBV-DNA and Hepatitis B e antigen [HBeAg] serostatus) were gathered from all patients.

2.3. Genetic screening

The PubMed database was thoroughly searched for relevant studies published from 1990 to December 2018 with the following keywords “hepatitis B virus X protein”, “HBx”, “signaling pathway”, “Hepatocellular Carcinoma”, and “HCC”. Ninety-two genes interacting with HBx or involving in signal pathways transactivated by HBx had been screened and included in this study (Table S1, Supplemental Digital Content, http://links.lww.com/MD/G391).

2.4. SNP selection

The 3'UTR sequences of these genes were obtained from UCSC database (http://genome.ucsc.edu). All SNPs in the 3'UTR with a minor allele frequency higher than 0.1 in Chinese population were identified through an extensive search in dbSNP database (https://www.ncbi.nlm.nih.gov/snp). Using the tools provided by the website (http://www.microrna.org/microrna/home.do) to identify SNPs at putative miRNA binding sites. The influence of these SNPs on the interaction between miRNAs and their targets were evaluated through calculating the change of minimum free energy (MFE, expressed in KJ/mol) between the 2 alleles through the online software-RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/).\cite{26} As proposed by Nicoloso et al,\cite{27} SNPs causing MFE changed more than 8% were considered biologically relevant and included in the study. As a result, 17 SNPs in 10 genes were selected for genotyping (Table 1).

2.5. DNA extraction and SNP genotyping

The genomic DNA was isolated from blood clot using TIANamp Blood Clor DNA Kit (Tiangen biotech Co. Ltd, Beijing, China) according to manufacturer’s protocol and stored at −80°C for genotyping. All samples were genotyped using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (Sequenom Inc., San Diego, CA). Amplification primers and extension primers were designed using the AssayDesigner3.1 software (Sequenom Inc.). Polymerase chain reaction (PCR) amplification of target sequence was performed in a multiplex reaction containing 1 μL genomic DNA. After PCR amplification, remaining dNTPs were dephosphorylated by shrimp alkaline phosphatase (Sequenom Inc.). Then, extension primers were used for locus-specific single-base extensions. The extension products were purified by cation-exchange resin (Sequenom Inc.), transferred onto the 384-well spectroCHIP (Sequenom Inc.) and genotyped using a matrix-assisted laser desorption ionization-time-of-flight mass spectrometer. Genotyping data were analyzed using the MassARRAYTyper software version 3.4 (Sequenom Inc.). The full research processes are shown in Figure 1.

2.6. Statistical analysis

Allele and genotype frequencies were calculated by counting for each locus. The deviation of these SNPs from the Hardy–Weinberg equilibrium was evaluated by Chi-square tests.
Table 1: The detailed information of SNPs in putative miRNA binding sites.  
| Gene   | SNP ID        | miRNA ID  | MFE (kJ/mol) | MFE change (%) |
|--------|---------------|-----------|--------------|----------------|
| AKT2   | rs33933140<sup>*</sup> | miR-216a  | 1.5          | 7.7            |
| AKT2   |               | miR-216b  | 1.6          | 9.5            |
| AKT2   |               | miR-490-3p| 2.0          | 8.0            |
| AKT3   | rs9428966     | miR-135a  | 0.7          | 3.7            |
| APC    | rs3733961<sup>*</sup> | miR-487b  | 1.8          | 12.5           |
| BIRC5  | rs2239680<sup>*</sup> | miR-335   | 4.1          | 27.7           |
| BIRC5  | rs1042489<sup>*</sup> | miR-708   | 0.7          | 3.7            |
| BIRC5  |               | miR-28-5p | 0.0          | 0.0            |
| BIRC5  |               | miR-132   | 1.0          | 5.0            |
| BIRC5  |               | miR-211-3p| 1.7          | -9.1           |
| BIRC5  |               | miR-7113-5p| 3.2        | -17.8          |
| BIRC5  |               | miR-505-5p| 3.9          | -21.5          |
| CTNNB1 | rs2053        | miR-296-3p| -1.8         | -7.7           |
| GRB2   | rs7219<sup>*</sup> | miR-326   | -2.8         | -13.6          |
| JAK3   | rs3008        | miR-1308  | 1.7          | -8.7           |
| KRAS   | rs712<sup>2</sup> | miR-422a  | -1.6         | -6.4           |
| NFKBIA | rs2273650<sup>*</sup> | miR-4459  | 1.1          | -5.8           |

(continued)
Continuous variables were expressed as means ± standard deviation and compared by t test. Categorical variables were assessed by Chi-square test. Logistic regression was performed to analyze the association between SNPs and HBV-related HCC using gender, age, HBV DNA, and HBeAg serostatus as covariates. Biological and clinical variables were compared between HBV-related HCC group and chronic HBV infection group by Student t test and Chi-square test for continuous and categorical variables, respectively. Statistical analysis was performed using the SPSS statistical software package version 19.0 (IBM Corp, Armonk, NY).

3. Results

3.1. Patient characteristics

In this study, 215 patients with HBV-related HCC and 216 patients with chronic HBV infection were recruited and assigned to HBV-related HCC group and chronic HBV infection group, respectively. Detailed characteristics of patients involved in this study are shown in Table 2. Serum albumin and cholinesterase representing liver synthesis were significantly lower in HBV-related HCC group than in the chronic HBV infection group (P < .01). Similarly, the proportion of patients with cirrhosis is significantly higher in HBV-related HCC than the group of chronic HBV infection (36.74% vs 15.74%, P < .001). The proportion of patients treated with nucleoside analogues was 14.88% in HBV-related HCC group, which was significantly lower than the patients in chronic HBV infection group (P < .001). The level of alpha-fetoprotein in HCC group was higher than the chronic HBV infection group (P < .001). The proportion of males, HBeAg positive, and drinking was not significantly different between HBV-related HCC group and chronic HBV infection group (Table 2).

3.2. Seventeen SNPs in 10 genes may affect the interaction between miRNAs binding with SNP sites and their targets

Out of 92 genes involved in this study, bioinformatics analysis identified 20 genes with 29 SNPs in the putative miRNA-binding sites. The detailed information of these 29 SNPs such as nucleotide variation, predicted binding miRNAs, MFE (expressed in KJ/mol) for each allele, and the change of MFE were shown in the Table 1. As listed in Table 1, 17 SNPs causing MFE changed more than 8% were selected for genotyping, another 12 SNPs were excluded. In order to evaluate whether this strategy was reasonable to choose functional SNPs, we searched electronic databases including PubMed, MEDLINE, ScienceDirect, Wiley, Web of Science, and Baidu Scholar for functional researches about all of these 29 SNPs. Out of the 17 SNPs included in genotyping, rs1042489, rs2239680, rs696, rs712, and rs9266 were confirmed to be functional in
previous researches. Meanwhile, none of the 12 SNPs excluded from genotyping were found to be functional. Therefore, bioinformatics analysis used in the present research was a reliable approach to identify functional SNPs.

3.3. Frequency and distribution of SNPs in HBV-related HCC group and chronic HBV infection group

The detailed allele frequencies and genotype distributions of these 17 SNPs in HBV-related HCC group and chronic HBV infection group were listed in Table 3. All of these SNPs were in Hardy–Weinberg equilibrium. Only found that rs1642742 (G>A) was associated with the occurrence of HBV-related HCC. The frequency of rs1642742 A allele in Von Hippel-Lindau (VHL) was significantly higher in HBV-related HCC group than in chronic HBV infection group \( P = 0.038, \text{ odds ratio [OR]} = 1.424, \) 95% confidence interval \([CI] = 1.019–1.899\) in dominant model, logistic regression revealed that heterozygotes of rs1642742 in VHL were less likely to be HCC than wild-type homozygotes. In dominant model, logistic regression revealed that patients with AA genotype versus AG and GG genotypes had an OR of 1.530 \( (P = 0.037, \text{ CI} = 1.025–2.286) \) for HBV-related HCC. Taken together, these results suggested that rs1642742 might increase the risk of HBV-related HCC and allele A might be a risk factor (Table 3).

3.4. Frequency and distribution of rs1642742 allele in HBV-related HCC patients

In the HCC group, some patients had tumor metastasis, while others did not. We further analyzed the genotype frequency and distribution of the 2 groups of patients with and without metastasis. Fifty-six patients (26.05%) with HBV-related HCC were found to have intra-hepatic or distant metastases, and 159 patients (73.95%) had not metastases. The frequency of rs1642742 A allele in VHL was significantly higher in metastasis group than in non-metastasis group \( P = 0.037, \text{ CI} = 1.031–3.895\) in dominant model, logistic regression revealed that patients with AA genotype versus AG and GG genotypes had an OR of 2.111 \( (P = 0.047, \text{ CI} = 1.001–4.456) \) for metastasis group. While the rs1642742 of VHL in dominant model and recessive model, logistic regression revealed that there were no differences between metastasis group and without metastasis group \( P > 0.05\). In summary, these results suggested that rs1642742 might be correlated with the risk of metastasis of HBV-related HCC as well, and AA genotype might be increased the risk of metastasis of HBV-related HCC (Table 4).

3.5. Regression analysis

The risk factors for metastasis of HBV-related HCC conferred by the combinations of age, gender, HBeAg, HBV-DNA, cirrhosis, alcohol consumption, antiviral with nucleoside analogues, and rs1642742 AA genotype were assessed using multivariate regression analysis. The results showed that the AA genotype of rs1642742 in HBV-related HCC patients was the only factor related with the cancer metastasis \( P = 0.038, \text{ OR} = 2.227, \) 95% CI \([1.043–4.732]\). There was no significant correlation between the other factors including the age more than 50years, male, HBeAg positive, HBV DNA \( \geq 10^5 \text{ copies/mL} \), cirrhosis, and alcohol consumption, and the metastasis of HCC (Fig. 2).

4. Discussion

During recent years, bioinformatics analysis was widely-used to detect SNPs that could interfere with the interaction between miRNAs and their targets.\textsuperscript{27} Consistent with previous researches, our study manifested that bioinformatics analysis was a reliable way to identify putative functional SNPs in miRNAs binding sites. Among 17 SNPs included in our case-control study, rs1042489,\textsuperscript{132} rs2273650,\textsuperscript{133} rs696,\textsuperscript{133,134} and rs712\textsuperscript{35} were involved in previous case-control studies. Consistent with previously reports, rs1042489, rs2273650, and rs696 were not associated with the risk of HCC in this study. However, rs712, which was found to increase the risk of HCC by Xiong et al.,\textsuperscript{35} was not associated with the risk of HCC in our study. A possible reason for this discrepancy might be that the cohort of this study was all chronic HBV infected patients, HBV infection was one of the most important risk factors for HCC as well-known,\textsuperscript{16} was not included in Xiong et al’s analyses. Although it is still difficult to give a better explanation, further research is needed to confirm...
The detailed allele frequencies and genotype distributions in 17 SNPs of patients.

| Genotype and allele | HBV-related HCC group (n = 215) | Chronic HBV infection group (n = 216) | P value |
|---------------------|---------------------------------|--------------------------------------|---------|
| rs1042489           |                                 |                                      |         |
| TT                  | 75 (37.69)                      | 64 (29.91)                           |         |
| CT                  | 92 (46.23)                      | 120 (56.07)                          |         |
| CC                  | 32 (16.08)                      | 30 (14.02)                           | .129    |
| T allele            | 242 (60.80)                     | 248 (57.94)                          |         |
| C allele            | 156 (39.20)                     | 180 (42.06)                          | .403    |
| (T+T+CT) vs CC     |                                 |                                      | .558    |
| TT vs (CT+CC)      |                                 |                                      | .094    |
| rs1042541           |                                 |                                      |         |
| GG                  | 81 (41.54)                      | 89 (41.59)                           |         |
| AG                  | 86 (44.10)                      | 105 (49.07)                          | .256    |
| AA                  | 28 (14.36)                      | 20 (9.35)                            |         |
| G allele            | 248 (63.59)                     | 283 (66.12)                          |         |
| A allele            | 142 (36.41)                     | 145 (33.88)                          | .449    |
| (G+G) vs AA        |                                 |                                      |         |
| GG vs (G+G+GA)     |                                 |                                      | .992    |
| rs1042542           |                                 |                                      |         |
| CC                  | 83 (42.35)                      | 89 (41.59)                           |         |
| CT                  | 83 (42.35)                      | 104 (48.60)                          | .185    |
| TT                  | 30 (15.31)                      | 21 (9.81)                            |         |
| C allele            | 249 (63.52)                     | 282 (65.89)                          |         |
| T allele            | 143 (36.48)                     | 146 (34.11)                          | .478    |
| (G+G) vs AA        |                                 |                                      | .092    |
| CC vs (CT+TT)      |                                 |                                      | .877    |
| rs1137188           |                                 |                                      |         |
| AA                  | 116 (59.49)                     | 135 (63.08)                          |         |
| AG                  | 70 (35.90)                      | 70 (32.71)                           |         |
| GG                  | 9 (4.62)                        | 9 (4.21)                             | .757    |
| A allele            | 302 (77.44)                     | 340 (79.44)                          | .486    |
| G allele            | 88 (22.56)                      | 88 (20.56)                           |         |
| (A+AA) vs GG       |                                 |                                      | .840    |
| AA vs (A+AA+GG)    |                                 |                                      | .936    |
| rs1642742           |                                 |                                      |         |
| AA                  | 133 (67.17)                     | 123 (67.21)                          |         |
| AG                  | 56 (28.28)                      | 78 (36.28)                           | .111    |
| GG                  | 9 (4.55)                        | 14 (6.51)                            |         |
| A allele            | 322 (81.31)                     | 324 (75.35)                          | .038    |
| G allele            | 74 (18.69)                      | 106 (24.65)                          | .384    |
| (A+AA) vs GG       |                                 |                                      | .037    |
| AA vs (A+AA+GG)    |                                 |                                      |         |
| rs2239680           |                                 |                                      |         |
| TT                  | 100 (51.28)                     | 120 (55.81)                          |         |
| CT                  | 81 (41.54)                      | 79 (36.74)                           | .606    |
| CC                  | 14 (7.18)                       | 16 (7.44)                            |         |
| T allele            | 281 (72.05)                     | 319 (74.19)                          | .491    |
| C allele            | 109 (27.95)                     | 111 (25.81)                          | .919    |
| (T+T+CT) vs CC     |                                 |                                      | .358    |
| TT vs (CT+CC)      |                                 |                                      |         |
| rs2273850           |                                 |                                      |         |
| CC                  | 113 (56.22)                     | 106 (49.30)                          |         |
| CT                  | 72 (35.82)                      | 99 (46.05)                           | .067    |
| TT                  | 16 (7.96)                       | 10 (4.65)                            |         |
| C allele            | 298 (74.13)                     | 311 (72.33)                          | .557    |
| T allele            | 104 (25.87)                     | 119 (27.67)                          | .164    |
| (C+CT) vs TT       |                                 |                                      | .158    |
| CC vs (CT+TT)      |                                 |                                      |         |
| rs2764805           |                                 |                                      |         |
| AA                  | 66 (33.17)                      | 66 (30.99)                           |         |
| AC                  | 91 (45.73)                      | 106 (49.77)                          |         |

(continued)
whether there is a correlation between HBV infection and rs712 polymorphism.

Association analysis indicated that rs1642742 might be associated with the risk of HBV-related HCC. Further stratification suggested that the allele G of rs1642742 might be a protective allele for patients while the allele A might be associated with tumorigenesis. According to our bioinformatics analysis, the variant allele of rs1642742, which is residing in the 3′UTR of the VHL gene, associated with a variety of benign and malignant tumors, may attenuate the expression of VHL via strengthen its binding with miRNAs. Mutation of VHL gene is associated with several types of tumors like clear-cell renal cancer, pancreatic cancer, pheochromocytoma,\textsuperscript{37–39} which suggests that VHL gene may play a role as a tumor suppressor in those cancers. Previous researches elucidated that VHL was able to form a ternary complex with elongin C and elongin B and induce proteasomal degradation of hypoxia-inducible factors.\textsuperscript{40} Moreover, recent study suggested that VHL negatively regulated antiviral signaling and affected innate antiviral immunity.\textsuperscript{41} Therefore, abnormal expression level of VHL caused by rs1642742 may contribute to the risk of HBV-related HCC.

Our results suggested that there was correlation between rs1642742 and metastasis and the allele A might be associated with tumor metastasis. Of course, the correlation between SNP and tumor metastasis had been reported in previous literatures, including lung cancer, breast cancer, gastric cancer, prostate cancer, and so on.\textsuperscript{42–45} Rs1642742 is residing in the 3′UTR of the VHL, which plays an important role in mRNA translocation, stability and translational regulation. The variant allele of rs1642742 may affect the ability of miRNA to bind to its sequence, which in turn affects the VHL gene expression and may lead to tumor metastasis. More researches should be conducted to further evaluate the effect of rs1642742 on the risk of HBV-related HCC and metastasis and clarify its possible mechanism.

Chronic HBV infection can cause chronic hepatitis, cirrhosis, liver failure, and HCC. Liver cirrhosis is an independent risk factor for HCC,\textsuperscript{46} which is obtained in this study as well. The high serum HBV-DNA level is closely related to the occurrence of HBV-related HCC, and reducing the viral-load can effectively lead to a decrease in the occurrence of HCC.\textsuperscript{47,48} In this study, the proportion of HCC patients with serum HBV-DNA levels <1 × 10⁵ copies/mL was significantly lower than that of patients with chronic HBV infection, which was consistent with the above studies. Long-term antiviral can delay the progression of the disease and reduce the incidence of serious endpoints, such as liver cirrhosis and HCC.\textsuperscript{49} Our research also suggests that antiviral HBV therapy may reduce the incidence of HCC.

### Table 3 (continued)

| Genotype and allele | HBV-related HCC group (n=215) | Chronic HBV infection group (n=216) | P value |
|--------------------|-----------------------------|----------------------------------|--------|
| GG vs (AG + AA)   | 116 (59.79) 135 (63.08)     | 70 (36.08) 70 (32.71)             | 0.772  |
| rs9266 GG         | 302 (77.84) 340 (79.44)     | 86 (22.16) 88 (20.56)             | 0.576  |
| rs958 C allele     | 124 (62.94) 127 (60.97)     | 61 (30.96) 80 (37.21)             | 0.495  |
| rs958 T allele     | 309 (78.43) 334 (77.67)     | 85 (21.57) 96 (22.33)             | 0.795  |

### Table 4

| Genotype and allele | Metastasis group (n=56) | Non-metastasis group (n=159) | P value |
|--------------------|------------------------|-------------------------------|--------|
| rs1642742 AA       | 40 (78.43) 93 (63.27)  | 10 (19.61) 46 (31.29)         | 0.127  |
| rs1642742 GG       | 1 (1.96) 8 (5.44)      | 12 (11.76) 62 (21.09)         | 0.037  |
| rs1642742 A allele  | 90 (88.24) 232 (78.91) | 88 (21.76) 96 (21.09)         | 0.304  |
| rs1642742 G allele  | 12 (11.76) 62 (21.09)  | 86 (22.16) 88 (20.56)         | 0.495  |

HBV = hepatitis B virus, HCC = hepatocellular carcinoma, SNP = single nucleotide polymorphism.
There are several limitations to our study. First of all, the sample size of our study is relatively small. Multicenter studies with a larger sample size are needed to further validate our findings. Secondly, the functional influence of these SNPs was predicted by bioinformatics analysis in our study. Thus, functional researches should be done to verify their function. Finally, besides SNPs in miRNAs binding sites, SNPs in the promoter region also influence the expression of a gene. Future researches investigating SNPs in the 3’UTR as well as SNPs in the 5’UTR will help us to better understand the mechanism of HBV-related HCC.

In conclusion, through bioinformatics analysis and case-control studies, we found that rs1642742 may be related to the occurrence and metastasis of HBV-related HCC for the first time. Although these results still need to be further validated, our findings provide clues for exploring the mechanism of HBV-related HCC.

Author contributions
(i) Xuebing Chen is acting as the submission’s guarantor (takes responsibility for the integrity of the work as a whole, from inception to published article).
(ii) Author contributions: Study concept and design: Xuebing Chen. Data curation: Hao Zhang, Xuebing Chen. Writing - original draft: Xuebing Chen, Hao Zhang. Writing - review & editing: Xuebing Chen.

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