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Impact of HBV infection on the association between HOTAIR SNPs and the risk of hepatocellular carcinoma: A mediation and interaction analysis

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Previous studies have demonstrated that single nucleotide polymorphisms (SNPs) rs12427129 and rs3816153 in HOX transcript antisense intergenic RNA (HOTAIR) might interact with hepatitis B virus (HBV) infection to increase the risk of hepatocellular carcinoma (HCC). However, it is unclear whether HBV infection is a potential mediator between HOTAIR rs12427129, rs3816153, and HCC. This study, including 1262 HCC cases and 1559 controls, aimed to use a four-way decomposition method to quantify the interaction and mediation effects of HBV infection in the association between rs12427129, rs3816153, and HCC. We found that rs12427129 and rs3816153 were associated with a risk of HBV infection among the controls (CC: CT+TT, adjusted odds ratio (OR)=1.77, 95% confidence interval (CI)=1.32–2.36 and GG: GT+TT, adjusted OR=0.63, 95% CI=0.48–0.82). The four-way decomposition revealed that rs12427129, rs3816153, and HBV infection had statistically significant reference interaction on HCC (excess risk (95% CI): –0.362 (–0.530, –0.195), p<0.001 and excess risk (95% CI): 0.433 (0.059, 0.808), p=0.023), and the proportion attributed to reference interaction were 110.82% and 125.27%, respectively. The pure indirect effect suggested that the rs3816153 GT + TT genotype can reduce the risk of HCC by 21.79% (excess risk (95% CI): –0.075 (–0.142, –0.009), p=0.026) when HBV infection as a mediator. Our findings suggested that HBV infection interacts or mediates with the association between rs12427129, rs3816153, and HCC. This would provide a new perspective for exploring the underlying biological mechanism between HOTAIR SNPs, HBV infection, and HCC.

Key words: hepatitis B virus, single nucleotide polymorphisms, hepatocellular carcinoma, four-way decomposition method

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and the third leading cause of cancer death worldwide [1]. According to estimates from the GLOBCAN 2018, there were 841,000 new HCC cases and 781,000 deaths worldwide, with China accounting for approximately 46.6% of the global cases [2, 3]. The high malignancy and rapid progress of HCC lead to the middle and late stages at the time of diagnosis in most HCC patients, and the prognosis of HCC remains poor, with less than a 15% five-year survival rate of HCC patients [4]. Therefore, it is necessary to explore the pathogenesis of HCC and identify individuals with an especially high risk of HCC to help prevent and detect HCC early.

The development of HCC is a complex multi-stage process that involves multiple risk factors, including hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, aflatoxin B1 intake, excessive alcohol consumption, and genetic alterations [4, 5]. Among them, HBV infection as an important risk factor for HCC [6], and long non-coding RNAs (lncRNAs) have been found to cause many genomic mutations related to the susceptibility of HCC [7]. HOX transcript antisense intergenic RNA (*HOTAIR*), a lncRNA with 2,158 nucleotides, is located in the HOXC cluster on the 12q13.13 region of the human chromosome [8]. Previous studies have demonstrated that *HOTAIR* is a carcinogenic gene in multiple cancers, including breast cancer [9], gastric cancer [10], and esophageal squamous cell carcinoma [11]. Regarding HCC, *HOTAIR* is highly expressed in HCC, and has been established as a predictor of progression and poor prognosis and a potential biomarker for lymph node metastasis in HCC [12]. *HOTAIR* potentially plays an oncogenic role through the interaction with miRNAs and decreasing the expression of SET domain-containing 2 (SETD2) [13]. When *HOTAIR* is overexpressed in HCC, it could play a vital

role in HBV-induced liver carcinogenesis by enhancing the ubiquitination of transcription repressor factors SUZ12 and zinc finger protein 198 (ZNF198) in the presence of polo-like kinase 1 (Plk1) [14]. However, the majority of studies that have focused on the relationship between *HOTAIR* or HBV infection and HCC have done so in isolation, without considering the complex interrelationships between *HOTAIR* and HBV infection, which may overlook the vital biological mechanism between *HOTAIR*, HBV infection, and HCC.

Single nucleotide polymorphisms (SNPs) are the most frequent genetic variation in the human genome, and SNPs occurring in functional regions may be associated with phenotype changes and disease susceptibility [15, 16]. Recently, an increasing number of studies have explored associations between *HOTAIR* SNPs and cancer risks [10, 17]. For example, the rs920778 in *HOTAIR* presented an increased association with gastric cancer risk [10], and the rs4759314 variant yielded a higher gastric cancer risk [18]. Additionally, the SNP rs920778 in *HOTAIR* was related to an increased risk of HCC [17]. We previously identified that two potentially functional *HOTAIR* SNPs (rs12427129 and rs3816153) and HBV infection have statistically significant interactions on HCC, rs12427129 and rs3816153 might exert the function of transcriptional regulation for *HOTAIR* expression [19]. However, what has been less clear is how much the interaction between HBV infection and *HOTAIR* rs12427129 or rs3816153 contributes to the potential biological mechanism of HCC, and whether HBV infection is a potential mediator of the association between *HOTAIR* rs12427129, rs3816153, and HCC. In-depth understanding and quantification of the role of HBV infection in the association between *HOTAIR* rs12427129, rs3816153, and HCC not only helps to clarify part of the mechanism of HCC at the molecular level but also provides new ideas for the prevention, treatment, and risk management of HCC.

In this study, we hypothesized that HBV infection status may mediate a biological causal effect along the path linking *HOTAIR* rs12427129 or rs3816153 with HCC. We applied the four-way decomposition method [20] to assess the role of mediation effects of HBV infection on the association between *HOTAIR* rs12427129, rs3816153, and HCC. Furthermore, we quantified the impact of the interaction between *HOTAIR* rs12427129, rs3816153, and HBV infection on HCC based on this method.

Patients and methods

Study population. This hospital-based case-control study included 1,262 newly diagnosed HCC cases and 1,559 control patients. All participants were recruited from the Shunde Hospital of Southern Medical University (Foshan, China) and the Cancer Center of Guangzhou Medical University (Guangzhou, China) from September 2010 to October 2014. All patients were diagnosed according to the diagnostic criteria of the "Diagnosis, management, and treatment of hepatocellular carcinoma (V2011)" [21]. The diagnosis was made through a combination of liver function tests, serum immunological markers, imaging examination (computed tomography or magnetic resonance imaging), and pathological confirmation. Patients were excluded if they were diagnosed with cancer other than HCC after the workup. Controls were frequency-matched to cases by age (± 5 years) and gender through randomly selecting healthy screened patients from the same hospital during the equivalent period as cases were enrollment. This study was approved by the Institutional Review Board of Guangdong Pharmaceutical University, and written informed consent was obtained from all participants before investigation.

Data collection. Epidemiological information for all subjects was collected by trained interviewers through in-person interviews, including gender, age (age at diagnosis for cases), drinking and smoking status, and family history of HCC in the first-degree relatives. Detail definitions were described previously [19]. Serological markers including HBsAg, anti-HBs, and anti-HBc were collected from the patients' medical records. The HBV antigen and antibody information of the control were detected by enzyme-linked immunosorbent assay (ELISA) kit (Kehua, Shanghai, China). For quality control, each reaction plate was set with negative, positive, blank, and repeated control. HBsAg positive was used as an indicator of HBV infection.

Candidate SNP selection and genotyping. SNPs covering regions from 2000 bp upstream to 2000 bp downstream of *HOTAIR* were retrieved from the 1000 Genomes Project for the Southern Chinese population. Bioinformatics tools such as SNP info, rVarBase, RegulomeDB, and HaploReg V4.1 were used to screen out potentially functional SNPs. A 5 ml peripheral blood sample was collected from each subject after the in-person interviews. Genomic DNA was extracted using the TIANamp DNA kit (Tiangen, Beijing, China) according to the manufacturer's protocol. All of these candidate SNPs were genotyped using the Sequenom MassARRAY iPLEX system (Sequenom, San Diego, CA, USA) according to the instructions of the manufacturer. To ensure the accuracy of genotyping, genotyping was performed without knowledge of subjects' disease status, and 5% of samples were randomly selected for repeated assays, with a concordance rate of 100%. Detailed candidate SNP selection and genotyping have been described elsewhere [19]. Based on previous research, rs12427129 C>T and rs3816153 G>T were selected for further analysis in this study.

Statistical analysis. Pearson's χ^2 tests were used to examine the differences in genotype distribution between non-HBV-infected and HBV-infected patients. Logistic regression models were fitted to investigate the associations between *HOTAIR* SNPs and HBV infection. Then the approach of four-way decomposition was used to analyze the interaction and mediation effects and their combination. Using this approach, we decomposed the total effect of HBV infection on the association between *HOTAIR* SNPs and HCC into

four components (Figure 1): controlled direct effect (CDE), reference interaction (INT_{ref}), mediated interaction (INT_{med}), and pure indirect effect (PIE). In the absence of HBV infection, the CDE of *HOTAIR* SNPs on HCC risk was the component of the total effect neither due to mediation nor due to interaction. The INT_{ref} of *HOTAIR* SNPs and HBV infection on HCC controlling for the effect of *HOTAIR* SNPs on HBV infection, which represented the component of the total effect due to interaction only (i.e., interaction rather than mediation). The INT_{med} only operated if the *HOTAIR* SNPs have an effect on the HBV infection, and this effect attributable to both mediation and interaction. The PIE of *HOTAIR* SNPs on HCC solely through HBV infection, which represented the component of the total effect that was due to only mediation (without any interaction). We further obtained the proportions of the effects that were attributable to each component by dividing the estimate of a component by the excess relative risk. Statistical analyses were implemented using SAS Version 9.4 (SAS Institute, Cary, NC, USA). All statistical tests were two-sided with a statistical significance of $p<0.05$. The 95% confidence intervals (95% CIs) of the excess risk in the four-way decomposition does not include zero, which was considered statistically significant.

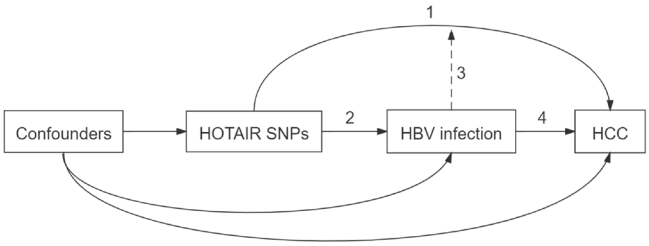

Figure 1. Conceptual framework of the four-way decomposition. Arrow 1 (without arrow 3): Controlled Direct Effect (CDE) of *HOTAIR* SNPs on HCC; Arrow 3+1 (without arrow 2): Reference Interaction (INT_{ref}) effect of *HOTAIR* SNPs and HBV infection on HCC; Arrow 2+4: Pure Mediation Effect (PIE) via HBV infection; Arrow 2+3+1: Mediated Interaction (INT_{med}) effect of *HOTAIR* SNPs and HBV infection on HCC. Confounders: age, gender, smoking status, drinking status, and family history of cancer.

Results

Characteristics of the study population. Characteristics of 1,262 HCC cases and 1,559 controls are presented in Supplementary Table S1. As described previously [19], the distributions of age and gender were similar in both groups (all $p>0.05$), but the differences in the distribution of smoking status, drinking status, and family history of cancer were statistically significant (all $p<0.05$).

Associations of *HOTAIR* SNPs and HBV infection with the risk of HCC. The genotype distributions of two SNPs in controls were all in agreement with Hardy-Weinberg equilibrium (all $p>0.05$; Supplementary Table S2). HBV infection was statistically associated with increased risk of HCC (adjusted OR=11.35, 95% CI: 9.42–13.67; Supplementary Table S1). Under the dominant model, compared to the CC genotype, carrying the rs12427129 CT/TT genotype has a protective effect on the risk of HCC (adjusted OR=0.72, 95% CI=0.57–0.90, p=0.005); while a significantly increased HCC risk was observed in carriers of the rs3816153 GT/TT genotypes (adjusted OR=1.30, 95% CI: 1.08–1.57, p=0.006) compared to those with the GG genotype (Supplementary Table S3).

Association between *HOTAIR* SNPs and HBV infection. Among the controls, significant differences were observed for rs12427129 and rs3816153 genotypes between non-HBV-infected and HBV-infected patients (all $p<0.001$). HBV-infected patients carried a higher proportion of rs12427129 CT+TT genotype or rs3816153 GG genotype than those without HBV infection. After adjusting for potential confounders, compared to the CC genotype, rs12427129 CT + TT genotypes were associated with an increased risk of HBV infection (OR=1.77, 95% CI=1.32–2.36, $p<0.001$). For rs3816153, compared to GG genotype, a protective effect of GT + TT genotype was found for HBV infection risk (OR=0.63, 95% CI=0.48–0.82, $p<0.001$) (Table 1). However, no significant association was observed between rs12427129 or rs3816153 and HBV infection in cases (Table 2).

Four-way decomposition. Table 3 showed the four-way decomposition of the effect of *HOTAIR* SNPs on HCC with HBV infection as the interaction/mediation variable. INT_{ref} and overall interaction were observed to be statistically significant in the association of rs12427129 and HBV infection

Table 1. Association between *HOTAIR* SNPs and HBV infection for controls.

Genotypes	Non-HBV infection n (%)	HBV infection n (%)	χ^2	p	Crude OR (95% CI)	p_{crude}	Adjusted OR (95% CI)	$p_{adjusted}$*
rs12427129			12.04	<0.001				
CC	990 (80.49)	229 (71.56)			1.00		1.00	
CT+TT	240 (19.51)	91 (28.44)			1.64 (1.24–2.17)	<0.001	1.77 (1.32–2.36)	<0.001
rs3816153			13.08	<0.001				
GG	741 (60.05)	228 (71.03)			1.00		1.00	
GT+TT	493 (39.95)	93 (28.97)			0.61 (0.47–0.80)	<0.001	0.63 (0.48–0.82)	<0.001

Abbreviations: CI – confidence interval; OR – odds ratio; HBV – hepatitis B virus; SNP – single nucleotide polymorphism. *Adjusted by age, gender, smoking status, drinking status, and family history of cancer.

Table 2. Association between *HOTAIR* SNPs and HBV infection for cases.

Genotypes	Non-HBV infection n (%)	HBV infection n (%)	χ^2	p	Crude OR (95% CI)	p_{crude}	Adjusted OR (95% CI)	$p_{adjusted}$*
rs12427129			0.21	0.644				
CC	258 (82.17)	754 (80.99)			1.00		1.00	
CT + TT	56 (17.83)	177 (19.01)			1.08 (0.78–1.51)	0.644	1.08 (0.77–1.51)	0.675
rs3816153			1.49	0.222				
GG	182 (57.23)	572 (61.11)			1.00		1.00	
GT + TT	136 (42.77)	364 (38.89)			0.85 (0.66–1.10)	0.223	0.83 (0.64–1.08)	0.16

Abbreviations: CI – confidence interval; OR – odds ratio; HBV – hepatitis B virus; SNP – single nucleotide polymorphism. *Adjusted by age, gender, smoking status, drinking status, and family history of cancer.

Table 3. Four-way decomposition of the effect of HBV infection on the association between HOTAIR rs12427129, rs3816153, and the risk of HCC.

Genotypes	Effect	Excess relative risk (95% CI)	p-value*	Proportion (%)
rs12427129	CDE	–0.015 (–0.066, 0.035)	0.055	4.68
(CC vs. CT + TT)	INT_{ref}	–0.362 (–0.530, –0.195)	<0.001	110.82
	INT_{med}	–0.039 (–0.083, 0.005)	0.085	11.92
	PIE	0.090 (–0.003, 0.183)	0.059	–27.42
	Overall Interaction	–0.402 (–0.591, –0.212)	<0.001	122.74
	Overall Mediated	0.051 (–0.005, 0.106)	0.072	–15.50
	Total Effect	–0.327 (–0.518, –0.136)	0.001	100.00
rs3816153	CDE	0.028 (–0.025, 0.081)	0.298	8.15
(GG vs. GT + TT)	INT_{ref}	0.433 (0.059, 0.808)	0.023	125.27
	INT_{med}	–0.040 (–0.090, 0.009)	0.112	–11.63
	PIE	–0.075 (–0.142, –0.009)	0.026	–21.79
	Overall Interaction	0.393 (0.052, 0.734)	0.024	113.64
	Overall Mediated	–0.115 (–0.222, –0.009)	0.034	–33.42
	Total Effect	0.346 (–0.013, 0.704)	0.059	100.00

Abbreviations: CI – confidence interval; OR – odds ratio; HBV – hepatitis B virus; CDE – controlled direct effect; INT_{ref} – reference interaction; INT_{med} – mediated interaction; PIE – pure indirect effect; Overall Interaction = INT_{ref} + INT_{med}; Overall Mediated = INT_{med} + PIE; Total Effect = CDE + INT_{ref} + INT_{med} + PIE = CDE + Overall Interaction + PIE = CDE + INT_{ref} + Overall Mediated. *Adjusted by age, gender, smoking status, drinking status, and family history of cancer.

with HCC (all p<0.001) overall. The excess risk of INT_{ref} was –0.362 (95% CI: –0.530, –0.195), and the proportion attributed to INT_{ref} was 110.82%. However, PIE is only marginally statistically significant (p=0.059). Further subgroup analysis by gender found that these effects were more pronounced in males than in females (Figure 2).

For rs3816153, PIE suggested that the GT + TT genotype can reduce the risk of HCC by 21.79% (excess risk (95% CI): –0.075 (–0.142, –0.009), p=0.026) when HBV infection was a mediator. INT_{ref} (excess risk (95% CI): 0.433 (0.059, 0.808), p=0.023) was also found in the impact of rs3816153 and HBV infection on HCC, and the proportion attributed to INT_{ref} was 125.27%. Besides, with HBV infection as the moderator and mediator, the overall interaction and overall mediated were also significant for the association between rs3816153 and HCC overall. Similarly, subgroup analysis by gender revealed that these effects were only statistically significant in males (Figure 2).

Discussion

In this study, we used a four-way decomposition method to decompose the interaction and mediation effects of HBV infection in the association between *HOTAIR* rs12427129, rs3816153 and HCC. We found that the excess risk of rs12427129 and rs3816153 on HCC was mainly explained by INT_{ref} (i.e. the pure interaction with HBV infection not caused by genetic variation of rs12427129 and rs3816153). More significantly, this study provided evidence to support that HBV infection can mediate the association between *HOTAIR* rs3816153 and HCC.

According to the functional annotations of RegulomeDB and rVarBase, rs12427129 is located in the transcription factor binding site (TFBS), the chromatin interaction region, and might affect the expression of *HOTAIR* by exerting transcriptional regulation. The rs3816153 located in the upstream region of *HOTAIR* affects promoter and enhancer

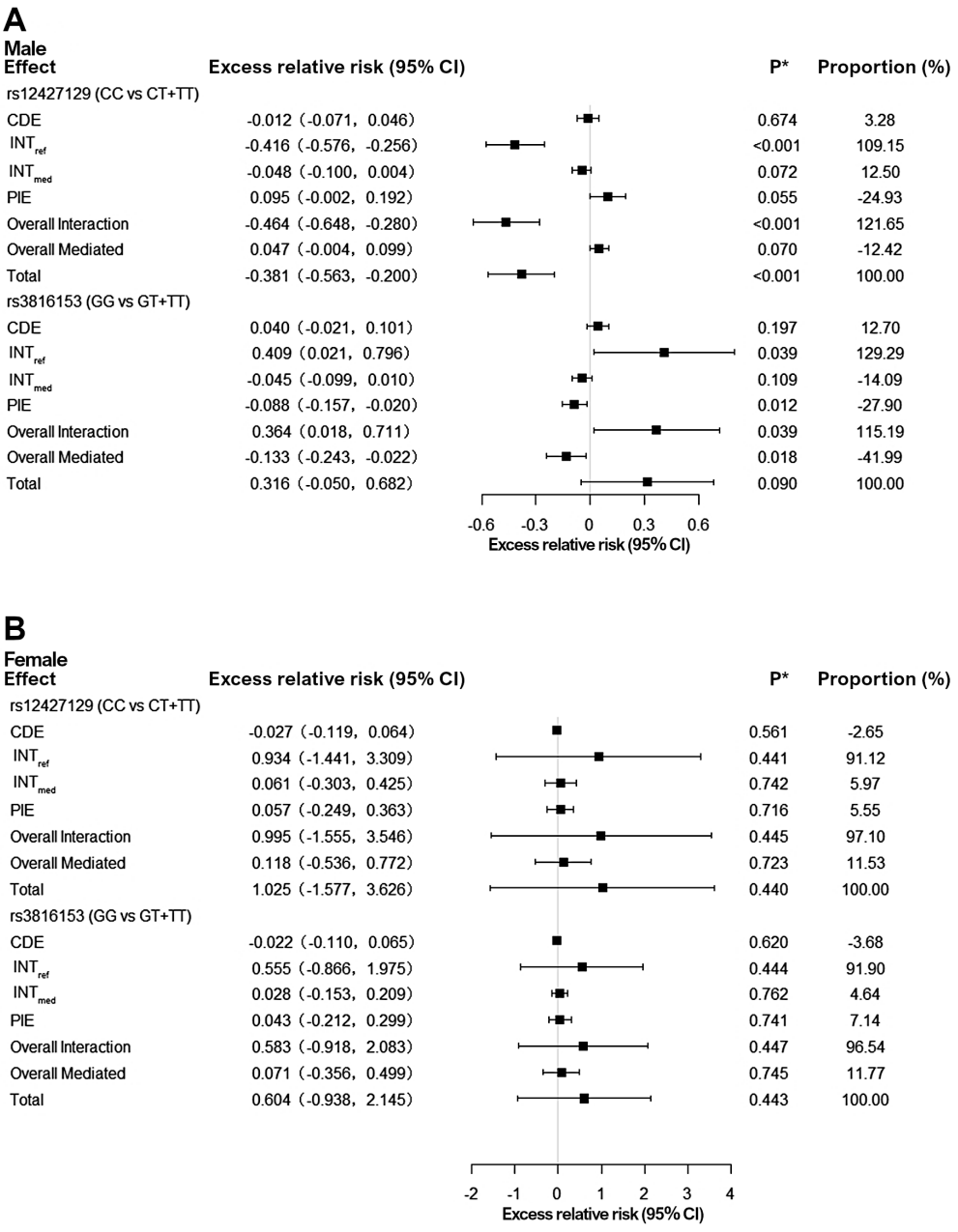

Figure 2. A gender subgroup analysis of the four-way decomposition effects. A) The four-way decomposition effects of HBV infection on the association between *HOTAIR* rs12427129, rs3816153, and HCC risk in males. B) The four-way decomposition effects of HBV infection on the association between *HOTAIR* rs12427129, rs3816153, and HCC risk in females. Abbreviations: CI – confidence interval; OR – odds ratio; HBV – hepatitis B virus; CDE – controlled direct effect; INT_{ref} – reference interaction; INT_{med} – mediated interaction; PIE – pure indirect effect; Overall Interaction = INT_{ref} + INT_{med}; Overall Mediated = INT_{med} + PIE; Total Effect = CDE + INT_{ref} + INT_{med} + PIE = CDE + Overall Interaction + PIE = CDE + INT_{ref} + Overall Mediated. *Adjusted by age, gender, smoking status, drinking status, and family history of cancer.

activity and regulates the gene expression of *HOTAIR* in specific cells and tissues, predicted by Haploreg. Evidence showed that *HOTAIR* can bind with E3 ubiquitin ligase Mex3B to antagonize the RNA helicase DEAD box protein 5 (DDX5), which plays a vital role in anti-HBV infection as a stabilizer of SUZ12 and a regulator of polycomb repressive complex 2 (PRC2) mediated gene repression [22]. In this way, *HOTAIR*-mediated the ubiquitination of SUZ12 released PRC2-mediated transcriptional repression, thereby promoting the transcription of HBV minichromosome and enhancing HBV replication, ultimately contributing to the occurrence of HCC [23]. These findings demonstrate that

HBV infection may act as a potential mediator of the association between *HOTAIR* SNPs and HCC. Additionally, SNP info, RegulomeDB, and rVarBase predicted that rs3816153 located in the TF binding region may affect microRNAs (miRNAs) binding activity or transcriptional regulation [24]. DDX5 has putative seed sequences of miRNAs belonging to miR-106b~25 and miR-17~92 clusters, and overexpression of these miRNAs may be the main reason for the downregulation of DDX5 [23]. Knocking down DDX5 can enhance the expression of HBV pre-genomic RNA (pgRNA) and nuclear covalently closed circular HBV-DNA (cccDNA). pgRNA is the mRNA of synthetic polymerase and core protein but also the template for HBV reverse transcription, and cccDNA is significant for the replication of HBV and the establishment of infection status [22, 25]. Therefore, *HOTAIR* rs3816153 may promote the establishment of HBV infection status by targeting miRNAs, which downregulate the DDX5, and finally contribute to the risk of HCC.

Interestingly, this study found that the direction of the interaction effect of rs12427129, rs3816153, and HBV infection are opposite to the mediation effect. The rs12427129 CC genotype and rs3816153 GT + TT genotype were dangerous genotypes that interact with HBV infection, but they were protective genotypes in the mediation effect. Combining the association between rs12427129, rs3816153, and HBV infection was stronger in controls than in cases, we can also presume that the impact of rs12427129 or rs3816153 on the risk of HBV infection mainly occurs in healthy people. Studies have shown that the same *HOTAIR* SNP genotype may have different effects on different outcomes [26, 27]. In breast cancer, individuals carrying the *HOTAIR* rs7958904 CC genotype have a higher risk of breast cancer than those carrying the GG genotype [26], while in osteosarcoma, subjects with the *HOTAIR* rs7958904 CC genotype have a significantly lower risk of osteosarcoma than those with GG genotype [27]. In addition, the same *HOTAIR* SNP genotype may play a different role in the development of the same disease [26, 28]. Lin *et al.* showed that *HOTAIR* rs7958904 CC genotype was significantly associated with the occurrence of breast cancer [26], while Zhou *et al.* found that *HOTAIR* rs7958904 CC genotype can reduce the risk of breast cancer cell proliferation, invasion, and metastasis [28]. Therefore, rs12427129 CC genotype and rs3816153 GT + TT genotype may reduce the risk of HBV infection in healthy people, but for those who already have HBV infection, rs12427129 CC genotype and rs3816153 GT + TT genotype maybe interacts with HBV infection to increase the risk of HCC.

In this study, we also found that the mediation and interaction effects of HBV infection in the association of rs12427129, rs3816153, and HCC mainly exist in men. There has been evidence showing the progression of HBV occurs faster in men than in women [29]. Androgens in men can directly bind to the androgen-responsive element (ARE) in the enhancer I (Enh I) of the HBV genome through signal transduction to enhance HBV replication and transcription, and it can further promote the development of HBV infections by upregulating HBV RNA transcription and inflammatory cytokines levels [30]. As stated above, there are potentially reasonable mechanisms for the interaction and mediation effects of HBV infection in the association of *HOTAIR* SNPs with HCC. Based on this, androgens in men may synergize with rs12427129 or rs3816153 to exert a key role in promoting HBV infection or interacting with HBV infection, and ultimately promote the occurrence and progression of HCC.

To the best of our knowledge, this study is the first utilizing the four-way decomposition to evaluate the interaction/mediation effects of HBV infection in the association between *HOTAIR* rs12427129, rs3816153, and HCC. Nevertheless, we should consider several limitations. Firstly, potential selection bias might occur because of the hospital-based design. Secondly, although many potential epidemiological factors were adjusted in this study, some residual confounding by some unmeasured factors (such as HCV status, diabetes, and metabolic syndrome) that are associated with rs12427129, rs3816153, HBV infection, and HCC cannot be excluded. Thirdly, this case-control study is restricted to Han ethnicity, which may affect the external validity of results.

In conclusion, our findings suggested that HBV infection may play an interaction/mediation role in the association between *HOTAIR* rs12427129, rs3816153, and HBV, especially in men. These findings highlight the etiological role of the *HOTAIR* SNPs and HBV infection in HCC development and provide a new perspective for exploring the underlying biological mechanism between *HOTAIR* SNPs, HBV infection, and HCC.

Supplementary information is available in the online version of the paper.

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Impact of HBV infection on the association between HOTAIR SNPs and the risk of hepatocellular carcinoma: A mediation and interaction analysis

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Supplementary Information

Supplementary Table S1. Characteristics of hepatocellular carcinoma cases and controls in the study

Characteristics	Cases (%) (n=1262)	Controls (%) (n=1559)	χ^2	Crude OR (95 % CI)	p_{crude}	Adjust OR (95 % CI)	$p_{adjusted}$ [a]
Gender			0.34		0.557		
Males	1092 (86.53)	1337 (85.76)		1.07 (0.86-1.32)		0.44 (0.33-0.58)	< 0.001
Females	170 (13.47)	222(14.24)		1.00		1.00	
Age (years)			1.69				
< 45	193 (15.29)	243 (15.59)		1.00		1.00	
45-60	531 (42.08)	619 (39.70)		1.08 (0.87-1.35)	0.405	1.34 (1.02-1.77)	0.154
> 60	538 (42.63)	697 (44.71)		0.97 (0.78-1.21)	0.262	1.35 (1.03-1.79)	0.117
Smoking status			176.32		< 0.001		< 0.001
Yes	773 (61.25)	559 (35.86)		2.83 (2.43-3.30)		2.25 (1.81-2.80)	
No	489 (38.75)	1000 (64.14)		1.00		1.00	
Drinking status			114.47		< 0.001		0.001
Yes	583(46.20)	418 (26.81)		2.34 (2.00-2.74)		1.54 (1.24-1.92)	
No	679(53.80)	1141 (73.19)		1.00		1.00	
Family history of cancers			5.13		0.023		0.454
Yes	212 (16.80)	214 (13.73)		1.27 (1.03-1.56)		1.10 (0.86-1.42)	
No	1050 (83.20)	1345 (86.27)		1.00		1.00	
HBV infection			828.70		< 0.001		< 0.001
Yes	944 (74.80)	321 (20.59)		11.45 (9.60-13.66)		11.35 (9.42-13.67)	
No	318 (25.20)	1238 (79.41)		1.00		1.00	

Abbreviations: CI-confidence interval; OR-odds ratio; HBV-hepatitis B virus

[a]Adjusting by age, gender, smoking status, drinking status, HBV infection and family history.

Supplementary Table S2. Functional prediction of SNPs in *HOTAIR* by Haploreg V4.1, SNP info, RegulomeDB, and rVarBase

rsID	Location	Location in gene	Allele	MAF in CHS	*P* for HWE	Miss cases and controls	Haploreg V4.1					
							Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	Selected eQTL hits
rs12427129	Chr12: 53973906	intronic	C/T	0.067	0.975	17/9	16 tissues	12 tissues including liver	21 tissues	5 bound proteins	-	1 hit
rs3816153	Chr12: 53976324	upstream	G/T	0.176	0.168	8/4	4 tissues including liver	8 tissues including liver	4 tissues	-	-	2 hits

rsID	SNP info			RegulomeDB				rVarBase			
	miRNA	Reg Potential	Conservation	scores	TF binding	DNase peak	eQTL	Related regulatory elements	Target genes	Extended variants	Associated traits
rs12427129	-	0.492	0.948	4	yes	yes	-	TFBR CIR	12	LD-proxies of rSNP Overlapped rCNV	-
rs3816153	Yes	0.294	0.913	1f	yes	yes	yes	TFBR CIR	13	LD-proxies of rSNP Overlapped rCNV	mRNA abundance

Abbreviations: SNP-single nucleotide polymorphism; MAF-minor allele frequency; CHS-Southern Han Chinese; HWE-Hardy-Weinberg equilibrium; TFBS-transcriptional factor binding site; eQTL-expression quantitative trait loci; TFBR-transcriptional factor binding region; CIR-Chromatin interactive region

Supplementary Table S3. Association analysis of the SNPs in *HOTAIR* and HCC susceptibility

Genotypes	Cases (%) (n=1262)	Controls (%) (n=1559)	χ^2	p	Crude OR (95 % CI)	p_{crude}	Adjusted OR (95 % CI)	$p_{Adjusted}$ [a]	*FDR*-$p_{Adjusted}$ [b]
rs12427129			4.89	0.087					
CC	1012 (81.29)	1219 (78.65)							
CT	210 (16.87)	309 (19.94)							
TT	23 (1.85)	22 (1.42)							
Dominant					0.85（0.70-1.02）	0.084	0.72（0.57-0.90）	0.005	0.009
rs3816153			1.59	0.452					
GG	754 (60.13)	969 (62.32)							
GT	442 (35.25)	513 (32.99)							
TT	58 (4.63)	73 (4.69)							
Dominant					1.10（0.94-1.28）	0.237	1.30（1.08-1.57）	0.006	0.009

Abbreviations: HCC-hepatocellular carcinoma; OR-odds ratio; CI-confidence interval; SNPs-single nucleotide polymorphisms

[a]Adjusted by age, gender, smoking status, drinking status, HBV infection, and family history of cancers

[b]$p_{adjusted}$ values for multiple comparison correction using the FDR method