Effect of functional nuclear factor-kappaB genetic polymorphisms on hepatitis B virus persistence and their interactions with viral mutations on the risk of hepatocellular carcinoma

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Background: Nonresolving inflammation and viral mutations are important in hepatitis B virus (HBV)-induced hepatocarcinogenesis. However, the effects of genetic polymorphisms affecting nuclear factor-kappaB (NF-κB) on HBV persistence and generation of hepatocellular carcinoma (HCC)-related HBV mutations remain unknown.

Patients and methods: rs28362491 (NFKB1 −941ns > Del), rs2233406 (NFKBIA −826C > T), rs3138053 (NFKBIA −881A > G), and rs696 (NFKBIA +2758G > A) were genotyped in 1342 healthy controls, 327 HBV-clearance subjects, and 3976 HBV-positive subjects including 1495 HCC patients, using quantitative PCR. HBV mutations were determined by sequencing. The NFKBIA promoter activity was assessed by transient transfection. Multiplicative interactions of the polymorphisms and viral mutations were assessed by multivariate logistic regression.

Results: Compared with HBV-clearance subjects, rs2233406 (CT versus CC) and rs3138053 (AG or AG + GG versus AA) significantly decreased HBV persistence, especially in the genotype B HBV-infected subjects. In the genotype C HBV-infected subjects, rs2233406 variant genotypes were significantly associated with an increased risk of HCC [CT versus CC: age-, gender-adjusted odds ratio (AOR), 1.33; 95% confidence interval (CI) 1.01–1.75 in training set and AOR, 1.59; 95% CI 1.01–2.52 in validation set] compared with HCC-free HBV-infected subjects and significantly increased the frequencies of HCC-related HBV mutations (A1762T/G1764A, T1753V, preS1 start codon mutation, and 3976 HBV-positive subjects including 1495 HCC patients, using quantitative PCR. HBV mutations were determined by sequencing. The NFKBIA promoter activity was assessed by transient transfection. Multiplicative interactions of the polymorphisms and viral mutations were assessed by multivariate logistic regression.

Conclusion: Genetic polymorphisms improving NF-κB activity contribute to genotype B HBV clearance. The rs2233406 variant genotypes significantly increased HCC risk in genotype C HBV-infected subjects, with AOR of 2.61 (95% CI 1.09–6.26).

Key words: NF-κB, polymorphism, hepatitis B virus, viral mutation, hepatocellular carcinoma, interaction

introduction

Hepatocellular carcinoma (HCC) accounts for 70%–85% of global liver cancer, one of the most common causes of cancer-related death worldwide. Chronic hepatitis B virus (HBV) infection remains a major cause of HCC worldwide [1]. In HBV-infected subjects, active hepatic inflammation, high HBV DNA level, HBV genotype, and some mutations in the enhancer II/basal core promoter/precore (EnhII/BCP/PC) and preS regions of HBV genome significantly increase HCC risk [2–5]. Nonresolving inflammation elicited by HBV infection may increase the HBV mutations via inducing the expression of cytidine deaminases [6]. Insufficient immunity selects the
HCC-related HBV mutations. These mutations, alone or in combination, in turn promote HCC [7, 8]. Thus, nonresolving inflammation is indispensable for the generation of HCC-risk HBV mutations, a process representing viral evolution in HBV-induced hepatocarcinogenesis.

Multiple signaling pathways are involved in human HCC development. Of those, nuclear factor-kappaB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) are important [9]. NF-κB is a collection of dimeric transcription factors. The major form of NF-κB in mammalian cells is a heterodimer of the p50 and p65 subunits. The p50 subunit, which is encoded by the *NFκB1* gene, has a –94 Del/Ins polymorphism in the promoter region. The –94 Del/Ins polymorphism has been associated with inflammatory disease and cancers [10–12]. In the cytoplasm, NF-κB is inactivated by binding to its inhibitor, the NFκBIA-encoded IkBα. The functional polymorphisms in the NFκBIA promoter and 3′ untranslated region (3′UTR) regions are significantly associated with cancers [12–14]. The impacts of these polymorphisms on HBV persistence and immune selection of HCC-related HBV mutations remain unknown.

In this study, we aimed to elucidate the effect of the functional NF-κB polymorphisms on HBV persistence, the generation of HCC-related HBV mutations, and their interactions with HBV mutations on HCC risk.

**patients and methods**

**study participants**

At training stage, healthy controls, hepatitis B surface antigen (HBsAg) naturally clearance subjects, asymptomatic HBsAg carriers (ASCs), patients with chronic hepatitis B (CHB), patients with liver cirrhosis (LC), and those with HCC were recruited from our community-based epidemiological survey in Shanghai and our collaborative hospitals in Shanghai, Shandong, Jiangsu, and Chongqing between September 2009 and October 2013. At validation stage, HBV-infected patients with or without HCC were enrolled from Beijing You-An Hospital between January 2009 and December 2012. Healthy controls, HBsAg naturally clearance subjects, and ASCs as well as CHB, LC, and HCC were defined or diagnosed as previously described [4, 15]. Subjects seropositive for antibodies against hepatitis C virus (HCV), hepatitis delta virus (HDV), and/or human immunodeficiency virus (HIV) were excluded. All participants were self-reported Han Chinese and provided written informed consent. The study protocol conformed to the ethical guidelines of the 2000 Declaration of Helsinki and was approved by the ethics committees of Second Military Medical University and Peking University Health Science Center.

**serological testing, HBV genotyping, and mutation analysis**

Fasting blood samples were obtained before clinical treatment. The serological markers of HBV and antibodies against HCV, HDV, and HIV were detected as previously described [15]. HBV DNA quantification, HBV genotyping, and liver function examination were carried out as previously described [4]. The EnhII/BCP/PC region and preS region of HBV genome were amplified by nested-PCR and directly sequenced [4, 5]. The wild-type nucleotides of each HBV genotype and HCC-related HBV mutations were determined as previously described [15].

**selection of NF-κB polymorphisms and genotyping**

Four representative single-nucleotide polymorphisms (SNPs) were selected in this study. rs28362491 (–94ins > Del) in the promoter region of *NFκB1* was selected because it affected the expression of *NFκB1* [10]. Functional SNPs rs2233406 (–826C > T) and rs3138053 (–881A > G) in the promoter region of *NFκBIA* were selected because they had been associated with HCC [12, 13]. rs696 (+2578G > A) in the 3′UTR of *NFκBIA* was selected because this SNP had been associated with colorectal cancer in different races [14]. These SNPs had a minor allele frequency of >11% in the Chinese Han population (http://www.hapmap.org/).

Primers and minor groove binder probes were designed and synthesized by GeneCore BioTechnologies (Shanghai, China). Primers and probes for genotyping these SNPs are listed in supplementary Table S1, available at *Annals of Oncology* online. Genomic DNA was extracted and SNPs were genotyped as previously described [15]. The successful genotyping rate was 97.4%, 97.1%, 97.1%, and 97.9% for rs2233406, rs3138053, rs696, and rs28362491, respectively.

**functional assay of NFκBIA promoter**

We amplified and sequenced *NFκBIA* promoter (nt.-1080-nt.-13) sequences from a male participant with –881A/–826C and another male participant with –881G/–826T. HBsAg gene (nt.1060-nt.1838) with and without A1762T/G1764A-based mutations were amplified and sequenced. Primers and conditions for amplifying *NFκBIA* promoter and HBsAg gene, construction of recombinant plasmids, transfection, and luciferase assay in nontumor hepatic cell line LO2 and HCC cell line HepG2 are provided in supplementary Table S2 and the supplementary Data, available at *Annals of Oncology* online.

**statistical analysis**

Hardy–Weinberg equilibrium (HWE) was examined online (http://ihg.gsf.de/ihg/snp.html). Student’s *t* test and *χ*^2^ test were used to calculate the difference for continuous variables and categorical variables, respectively. For the effects of NF-κB SNPs on HBV persistence, the frequencies of HBV mutations, and HCC risk, an unconditional logistic regression model was conducted to calculate odds ratios and their 95% confidence intervals (CIs), adjusting for age and gender. The multiplicative interactions of SNPs with HBV mutations for the HCC development were tested by multivariate logistic regression analysis, adjusting for age and gender. All statistical tests were two sided and conducted by SPSS 16.0 for Windows (SPSS, Chicago, IL). A *P* value of <0.05 was considered statistically significant.

**results**

**characteristics of study participants**

A total of 5645 participants were enrolled in this study. The characteristics of these participants are listed in supplementary Table S3, available at *Annals of Oncology* online. In training set with 4515 participants, age was matched among HCC patients, healthy controls, and HCC-free HBV-infected subjects. No significant difference was found in HBV DNA level between HCC patients and HCC-free HBV-infected subjects. In validation set, these variables were not matched between HCC-free HBV-infected patients and HBV–HCC patients.

**associations of NF-κB SNPs with HBV chronic infection and HCC risk**

In control populations (healthy controls and HBsAg clearance subjects), the four SNPs were conform to HWE (*P* > 0.05 for
The associations of NF-κB SNPs with HBV persistence were evaluated in training set (Table 1). In genotype B HBV-infected subjects, rs3138053 AG genotype and G (AG + GG) allele were significantly associated with decreased risks of HBV persistence compared with HBV natural clearance subjects, this effect was still significant after Bonferroni correction ($P = 0.016$ and $P = 0.010$, respectively). In the genotype C HBV-infected subjects, rs3138053 G (AG + GG) allele was associated with a decreased risk of HBV persistence compared with HBV-clearance subjects ($P = 0.048$). Thus, the variant polymorphic genotypes in the promoter region of NFKBIA gene may facilitate HBV clearance, especially in genotype B HBV-infected subjects.

In the genotype C HBV-infected subjects, rs2233406 CT genotype and T (CT + TT) allele were significantly associated with increased risks of HCC compared with HBV-free controls ($P = 0.027$ and $P = 0.036$, respectively); rs2233406 CT genotype was associated with an increased risk of HCC compared with HCC-free HBV-infected subjects ($P = 0.043$) in training set. This effect could be repeated in validation set (Table 2). However, these SNPs were not associated with HCC in the genotype B HBV-infected subjects (supplementary Table S4, available at *Annals of Oncology* online).

### associations of NF-κB SNPs with frequencies of the HCC-related HBV mutations

We successfully sequenced the EnhII/BCP/PC region and the preS region from 1450 (50.95%) and 1285 (45.15%) of the HBV-infected subjects in training set, respectively (GenBank accession No. JX556943-JX559050, KC934199-KC934744, and KJ019219-KJ019299). rs2233406 variant genotypes were significantly associated with increased frequencies of HCC-risk HBV mutations (T1753V, A1762T/G1764A, G1719T, preS deletion, and preS1 start codon mutation) in genotype C HBV-infected subjects. The variant genotypes of rs28362491 were significantly associated with an increased frequency of A1762T/G1764A and a decreased frequency of preS2 start codon mutation in genotype C HBV-infected subjects. rs28362491 Del/Del genotype facilitated the generation of G1899A, a HCC-risk HBV mutation [4], in genotype B HBV-infected subjects. rs696 and rs3138053...

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**Table 1.** Association of NF-κB polymorphisms with HBV persistence/natural clearance

<table>
<thead>
<tr>
<th>NF-κB SNP</th>
<th>SNP genotype</th>
<th>Healthy control</th>
<th>HBsAg clearance subjects</th>
<th>HCC-free HBV-infected subjects</th>
<th>AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HCC-free HBV-infected subjects versus healthy controls</td>
</tr>
<tr>
<td>In genotype B HBV-infected subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs696</td>
<td>GG</td>
<td>446</td>
<td>115</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>(NFKBIA +2758G &gt; A)</td>
<td>GA</td>
<td>649</td>
<td>145</td>
<td>109</td>
<td>0.89 (0.65–1.21)</td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>850</td>
<td>192</td>
<td>164</td>
<td>1.02 (0.76–1.36)</td>
</tr>
<tr>
<td>rs2233406</td>
<td>CC</td>
<td>1048</td>
<td>244</td>
<td>204</td>
<td>1</td>
</tr>
<tr>
<td>(NFKBIA −826C &gt; T)</td>
<td>CT</td>
<td>244</td>
<td>77</td>
<td>41</td>
<td>0.84 (0.58–1.22)</td>
</tr>
<tr>
<td></td>
<td>CT + TT</td>
<td>268</td>
<td>81</td>
<td>47</td>
<td>0.88 (0.62–1.25)</td>
</tr>
<tr>
<td>rs3138053</td>
<td>AA</td>
<td>1056</td>
<td>233</td>
<td>214</td>
<td>1</td>
</tr>
<tr>
<td>(NFKBIA −881A &gt; G)</td>
<td>AG</td>
<td>234</td>
<td>74</td>
<td>38</td>
<td>0.80 (0.55–1.16)</td>
</tr>
<tr>
<td></td>
<td>AG + GG</td>
<td>256</td>
<td>79</td>
<td>39</td>
<td>0.74 (0.51–1.08)</td>
</tr>
<tr>
<td>rs28362491</td>
<td>ins/ins</td>
<td>432</td>
<td>117</td>
<td>97</td>
<td>1</td>
</tr>
<tr>
<td>(NFKB1 −94Ins &gt; Del)</td>
<td>ins/del</td>
<td>653</td>
<td>152</td>
<td>127</td>
<td>0.90 (0.67–1.21)</td>
</tr>
<tr>
<td></td>
<td>ins/del + del/del</td>
<td>873</td>
<td>208</td>
<td>155</td>
<td>0.82 (0.62–1.08)</td>
</tr>
<tr>
<td>In genotype C HBV-infected subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs696</td>
<td>GG</td>
<td>446</td>
<td>115</td>
<td>233</td>
<td>1</td>
</tr>
<tr>
<td>(NFKBIA +2758G &gt; A)</td>
<td>GA</td>
<td>649</td>
<td>145</td>
<td>331</td>
<td>0.96 (0.78–1.19)</td>
</tr>
<tr>
<td></td>
<td>GA + AA</td>
<td>850</td>
<td>192</td>
<td>457</td>
<td>1.01 (0.82–1.23)</td>
</tr>
<tr>
<td>rs2233406</td>
<td>CC</td>
<td>1048</td>
<td>244</td>
<td>547</td>
<td>1</td>
</tr>
<tr>
<td>(NFKBIA −826C &gt; T)</td>
<td>CT</td>
<td>244</td>
<td>77</td>
<td>130</td>
<td>1.00 (0.78–1.27)</td>
</tr>
<tr>
<td></td>
<td>CT + TT</td>
<td>268</td>
<td>81</td>
<td>142</td>
<td>1.00 (0.79–1.26)</td>
</tr>
<tr>
<td>rs3138053</td>
<td>AA</td>
<td>1056</td>
<td>233</td>
<td>550</td>
<td>1</td>
</tr>
<tr>
<td>(NFKBIA −881A &gt; G)</td>
<td>AG</td>
<td>234</td>
<td>74</td>
<td>131</td>
<td>1.06 (0.83–1.35)</td>
</tr>
<tr>
<td></td>
<td>AG + GG</td>
<td>256</td>
<td>79</td>
<td>139</td>
<td>1.03 (0.81–1.30)</td>
</tr>
<tr>
<td>rs28362491</td>
<td>ins/ins</td>
<td>432</td>
<td>117</td>
<td>238</td>
<td>1</td>
</tr>
<tr>
<td>(NFKB1 −94Ins &gt; Del)</td>
<td>ins/del</td>
<td>653</td>
<td>152</td>
<td>342</td>
<td>0.99 (0.80–1.22)</td>
</tr>
<tr>
<td></td>
<td>ins/del + del/del</td>
<td>873</td>
<td>208</td>
<td>458</td>
<td>0.98 (0.80–1.20)</td>
</tr>
</tbody>
</table>

*The association was still significant after Bonferroni correction with a cutoff $P$ value of 0.025.

AOR, adjusted odds ratio (adjusted for age and gender); CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; SNP, single-nucleotide polymorphism.
were not statistically associated with the HBV mutations in both genotypes. These data are shown in Table 3.

We further analyzed the frequencies of important HCC-related HBV mutations in ASCs, CHB patients, LC patients, and HCC patients with different rs2233406 alleles or rs28362491 genotypes (supplementary Figure S1, available at Annals of Oncology online). In the genotype C HBV-infected subjects, the frequencies of A1762T/G1764A and T1753V consecutively in both cell lines, while HBx, either with wild-type HBx and co-transfected with mutated HBx containing A1762T/G1764A-based mutations in both cell lines (supplementary Figure S2, available at Annals of Oncology online).

**Table 2. Association of NF-κB polymorphisms with the risk of HCC in genotype C HBV-infected subjects**

<table>
<thead>
<tr>
<th>NF-κB SNP</th>
<th>SNP genotype</th>
<th>HBV-free controls*</th>
<th>HCC-free HBV-infected subjects</th>
<th>HCC-controla</th>
<th>AOR (95% CI)</th>
<th>HBV–HCC versus HBV-free controls</th>
<th>HBV–HCC versus HBV-free infected subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training set</td>
<td>rs696</td>
<td>GG</td>
<td>554</td>
<td>233</td>
<td>220</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(NFκBIA +2758G &gt; A)</td>
<td>GA</td>
<td>785</td>
<td>331</td>
<td>291</td>
<td>0.99 (0.80–1.22)</td>
<td>0.95 (0.74–1.21)</td>
<td></td>
</tr>
<tr>
<td>rs2233406</td>
<td>GA + AA</td>
<td>1027</td>
<td>457</td>
<td>398</td>
<td>1.03 (0.84–1.25)</td>
<td>0.96 (0.76–1.22)</td>
<td></td>
</tr>
<tr>
<td>(NFκBIA −826C &gt; T)</td>
<td>CT</td>
<td>1271</td>
<td>547</td>
<td>465</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>rs3138053</td>
<td>CT + TT</td>
<td>318</td>
<td>130</td>
<td>148</td>
<td>1.29 (1.03–1.63)</td>
<td>1.33 (1.01–1.75)</td>
<td></td>
</tr>
<tr>
<td>(NFκBIA −881A &gt; G)</td>
<td>A1762T/G1764A</td>
<td>1269</td>
<td>550</td>
<td>487</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>rs28362491</td>
<td>A1762T/G1764A</td>
<td>304</td>
<td>131</td>
<td>122</td>
<td>1.06 (0.83–1.34)</td>
<td>1.11 (0.84–1.47)</td>
<td></td>
</tr>
<tr>
<td>(NFκB1 −94Ins &gt; Del)</td>
<td>AG</td>
<td>331</td>
<td>139</td>
<td>131</td>
<td>1.04 (0.82–1.31)</td>
<td>1.11 (0.84–1.46)</td>
<td></td>
</tr>
<tr>
<td>rs28362491</td>
<td>AG + GG</td>
<td>542</td>
<td>238</td>
<td>205</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ins/del</td>
<td>790</td>
<td>342</td>
<td>312</td>
<td>1.09 (0.89–1.35)</td>
<td>1.06 (0.82–1.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ins/del + del/del</td>
<td>1064</td>
<td>458</td>
<td>419</td>
<td>1.07 (0.87–1.31)</td>
<td>1.07 (0.85–1.36)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Healthy controls plus HBsAg clearance subjects.
AOR, adjusted odds ratio (adjusted for age and gender); CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.

**discussion**

In this study, the effect of NF-κB SNPs on HBV persistence was firstly evaluated. We found that the variant genotypes of NFκBIA -881 and/or -826 SNPs were inversely associated with HBV persistence. The NFκBIA -881A-826C promoter were significantly more active than those of the -881G-826T counterpart in both cell lines, while HBx, either with A1762T/G1764A-based mutations or without, significantly increased the activities of NFκBIA -881A-826C promoter and the -881G-826T counterpart. However, we did not find apparent difference in the activities of NFκBIA promoter co-transfected with wild-type HBx and co-transfected with mutated HBx containing A1762T/G1764A-based mutations in both cell lines (supplementary Figure S2, available at Annals of Oncology online).
inflammation than genotype C; whereas genotype C often leads to higher persistence following an acute infection and is more apt to cause LC and HCC than genotype B [2, 3, 16, 17]. NF-κB can inhibit HBV replication via MyD88, a key molecule in the signaling cascade of innate immune response [18]. Active inflammation in response to NF-κB expression might play a key role in promoting immune clearance of genotype B HBV.

In the genotype C HBV-infected subjects, rs2233406 variant genotype and allele were significantly associated with increased risks of HCC compared with HBV-free controls or HCC-free HBV-infected subjects (Table 2). These results are consistent with our previous study conducted in a different population [13]. rs28362491 (NFKB1 −94Ins > Del) was not statistically associated with HCC risk, which is different from a study carried out in Taiwan [12]. This disparity might be caused by different HBV genotypes endemic in the two areas. Interestingly, rs3138053 was not statistically associated with HCC risk.

### Table 3. Significant associations of the NF-κB polymorphisms with the frequencies of the HCC-risk HBV mutations

<table>
<thead>
<tr>
<th>NF-κB SNP</th>
<th>Genotype C HBV-infected subjects</th>
<th>Genotype B HBV-infected subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBV mutation</td>
<td>AOR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2233406</td>
<td>T1753V</td>
<td>2.09 (1.47–2.99)</td>
</tr>
<tr>
<td></td>
<td>A1762T/G1764A preS deletion</td>
<td>1.55 (1.08–2.22)</td>
</tr>
<tr>
<td></td>
<td>G1719T preS1 start codon mutation</td>
<td>4.53 (1.02–20.05)</td>
</tr>
<tr>
<td></td>
<td>T1753V</td>
<td>1.92 (1.36–2.72)</td>
</tr>
<tr>
<td></td>
<td>A1762T/G1764A preS1 start codon mutation</td>
<td>1.49 (1.05–2.11)</td>
</tr>
<tr>
<td>rs28362491</td>
<td>Ins/Ins</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>preS2 start codon mutation</td>
<td>0.60 (0.42–0.85)</td>
</tr>
<tr>
<td></td>
<td>A1762T/G1764A preS2 start codon mutation</td>
<td>1.62 (1.07–2.46)</td>
</tr>
<tr>
<td></td>
<td>G1899A</td>
<td>3.12 (1.01–9.60)</td>
</tr>
</tbody>
</table>

AOR, adjusted odds ratio (adjusted for age and gender); CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.

### Table 4. Interactions of NF-κB polymorphisms with the HBV mutations on the risk of HCC in the genotype C HBV-infected subjects

<table>
<thead>
<tr>
<th>NF-κB SNP</th>
<th>HBV mutations</th>
<th>HCC-free HBV-infected subjects</th>
<th>HBV–HCC</th>
<th>AOR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2233406</td>
<td>A1762T/G1764A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>AG/AA/TG</td>
<td>216</td>
<td>73</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>TA</td>
<td>174</td>
<td>223</td>
<td>3.52 (2.50–4.95)</td>
<td>4.21 × 10−13</td>
</tr>
<tr>
<td>CC + TT</td>
<td>AG/AA/TG</td>
<td>51</td>
<td>9</td>
<td>0.50 (0.23–1.07)</td>
<td>0.073</td>
</tr>
<tr>
<td>CC + TT</td>
<td>TA</td>
<td>46</td>
<td>85</td>
<td>2.16 (1.72–2.72)</td>
<td>4.10 × 10−11</td>
</tr>
<tr>
<td>For interaction</td>
<td></td>
<td></td>
<td></td>
<td>2.61 (1.09–6.26)</td>
<td>0.032</td>
</tr>
<tr>
<td>rs2233406</td>
<td>preS2 start codon mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>289</td>
<td>213</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1</td>
<td>55</td>
<td>96</td>
<td>2.23 (1.52–3.28)</td>
<td>4.45 × 10−5</td>
</tr>
<tr>
<td>CC + TT</td>
<td>0</td>
<td>85</td>
<td>89</td>
<td>1.44 (1.00–2.06)</td>
<td>0.048</td>
</tr>
<tr>
<td>CC + TT</td>
<td>1</td>
<td>20</td>
<td>22</td>
<td>1.14 (0.82–1.58)</td>
<td>0.429</td>
</tr>
<tr>
<td>For interaction</td>
<td></td>
<td></td>
<td></td>
<td>0.42 (0.19–0.93)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

AOR, adjusted odds ratio (adjusted for age and gender); CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.
rs2233406 and rs3138053 are in complete linkage disequilibrium with each other in healthy populations. From our results (Table 2), the two SNPs might not be in complete linkage disequilibrium in the HBV-infected population. In the genotype C HBV-infected subjects, rs2233406 SNP-mediated constitutive activation of NF-κB may promote the nonresolving inflammation, a prerequisite for HBV-induced hepatocarcinogenesis.

For the first time, we found that rs2233406 variant genotypes facilitated the generation of A1762T/G1764A, T1753V, preS1 start codon mutation, and preS deletion, the HCC-risk HBV mutations; rs28362491 Del/Del genotype was significantly associated with an increased frequency of A1762T/G1764A and a decreased frequency of preS2 start codon mutation. Interestingly, A1762T/G1764A and T1753V were significantly higher in the genotype C HBV-infected HCC patients with rs2233406 T allele than in those with rs2233406 C allele. Thus, the immune selection milieu predisposed by rs2233406 polymorphism might be special in those who are more likely to develop HCC. A1762T/G1764A can also be preferably selected in HBV-infected subjects with the HCC-risk genotypes of rs2293152 in STAT3 pathway [19]. Genetic predisposition of these important signaling molecules facilitating nonresolving inflammation contribute greatly to the immune selection of the HBV mutations. The NF-κB SNPs preferably affected the HBV mutations at the 3’-terminal of HBx (Table 3), indicating possible link between HBx and NF-κB. Interplay between HBx and NF-κB signaling is important in HBV-induced hepatocarcinogenesis [20]. As HBx containing A1762T/G1764A-based mutations promote the malignant phenotypes of HCC cells [7], the effect of rs2233406 polymorphism on HCC risk might be, at least partially, caused by the HBV mutations. We found that the interaction of rs2233406 variant genotypes and A1762T/G1764A significantly increased HCC risk in genotype C HBV-infected subjects (Table 4). This is possibly caused by that rs2233406 variant genotypes facilitate the generation of HCC-risk mutations including A1762T/G1764A because we did not find clear evidence indicating that A1762T/G1764A-containing HBx significantly transacted the activity of NFKBIA promoter with rs2233406 variant genotypes both in hepatic and HCC cells (Supplementary Figure S2, available at Annals of Oncology online). Nevertheless, these interactions should be of importance in identifying HBV-infected subjects who are more likely to develop HCC.

To the best of our knowledge, this is the first study reporting that NF-κB genetic polymorphisms influence immune selection of HCC-risk HBV mutations and affect HCC risks via interacting with these mutations. Moreover, a large sample size allowed for multiple stratifications and provided convincing data. However, our study had limitations. First, we only amplified the two HBV fragments from half of the HBV-infected subjects, resulting in a possible preponderance of missing data. Secondly, other environmental exposures such as dietary glycemic load were incomplete and therefore not included. Dietary glycemic load affects HCC risk in the subjects with or without chronic hepatitis [21].

conclusion

The variant genotypes of the polymorphisms at NFKBIA promoter region may predispose the host to clear genotype B HBV preferentially. The variant genotypes of rs2233406 impair the expression of IxBo and facilitate immune selection of the HCC-related HBV mutations, therefore increasing the risk of HCC in chronic HBV-infected subjects. This study provides some insights of the host–virus interactions in HBV-induced hepatocarcinogenesis and should be important in identifying HBV-infected subjects who are more likely to develop HCC.

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disclosure

The authors have declared no conflicts of interest.

references

Glucocorticoid prescriptions and breast cancer recurrence: a Danish nationwide prospective cohort study

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Background: Treatment with synthetic glucocorticoids (GCs) depresses the immune response and may therefore modify cancer outcomes. We investigated the association between GC use and breast cancer recurrence.

Materials and methods: We conducted a population-based cohort study to examine the risk of breast cancer recurrence associated with GC use among incident stage I–III female breast cancer patients aged >18 years diagnosed 1996–2003 in Denmark. Data on patients, clinical and treatment factors, recurrence, and comorbidities as well as data on GC prescriptions and potential confounders were obtained from Danish population-based medical registries. GCs were categorized according to administrative route: systemic, inhaled, or intestinal. Women were followed for up to 10 years or until 31 December 2008. We used Cox proportional hazards regression models to compute hazard ratios (HRs) and associated 95% confidence intervals (95% CIs) to evaluate the association between GC use and recurrence. Time-varying drug exposures were lagged by 1 year.

Results: We included 18,251 breast cancer patients. Median recurrence follow-up was 6.9 years; 3,408 women developed recurrence during follow-up. Four thousand six hundred two women filled at least one GC prescription after diagnosis. In unadjusted models, no association was observed among users of systemic, inhaled, and intestinal GCs (HRsystemic = 1.1, 95% CI 0.9–1.3; HRinhaled = 0.9, 95% CI 0.7–1.0; and HRintestinal = 1.0, 95% CI 0.9–1.2) versus nonusers. In adjusted models, the results were also near null (HRsystemic = 1.1, 95% CI 0.9–1.2; HRinhaled = 0.8, 95% CI 0.7–1.0; and HRintestinal = 1.0, 95% CI 0.8–1.2).

Conclusion: We found no evidence of an effect of GC use on breast cancer recurrence.

Key words: breast neoplasm, glucocorticoids, outcome, epidemiology

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