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ORIGINAL RESEARCH

Impact of HBV Integration on Hepatocellular Carcinoma After Long-Term Antiviral Therapy

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Purpose: Few studies have reported the integrated characteristics of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) after long-term antiviral therapy. This study aimed to investigate the HBV integration features in HBV-HCC patients who had undergone long-term antiviral therapy, evaluate their impact on clinical indicators, and analyze the potential mechanisms involved.

Patients and Methods: We utilized genome-wide association study (GWAS) to analyze liver cancer tissues and detect the presence of HBV integration. Seventeen patients with HBV integration were included in the integration (Int) group, while the remaining five patients were included in the non-integration (N-int) group. Clinical indicators were regularly monitored and compared between the two groups. The characteristics of HBV integration patterns were analyzed, and differences between the groups were explored at the chromosome and genomic levels.

Results: After long-term antiviral therapy, although the frequency of HBV integration in HBV-HCC was reduced, residual HBV integration still accelerated the development of HCC. It affected the diagnosis, treatment, and prognosis of patients. HBV integration events led to changes in chromosome structure, which were closely related to HCC. Novel fusion genes were detected at a high frequency and had the potential to be specific detection sites for HBV-HCC.

Conclusion: HBV integration events are synergistically involved in the human genome and HBV, which can lead to chromosome structural instability, gene rearrangement events closely related to HCC production, and the formation of new specific fusion genes.

Keywords: hepatitis B virus, long-term antiviral therapy, hepatocellular carcinoma, integration, gene rearrangement

Introduction

Primary liver cancer (PLC) is the third most common cause of cancer-related deaths worldwide.[1,2] The predominant histological type of PLC is hepatocellular carcinoma (HCC).[3] Factors contributing to HCC occurrence include hepatitis virus infection, long-term excessive alcohol consumption, and chronic exposure to aflatoxin. Notably, hepatitis B virus (HBV) infection is a significant factor leading to HCC,[4–6] particularly in China, where approximately 80% of HCC cases are associated with HBV infection.[7,8] Despite the widespread use of nucleos(t)ide analogues (NUCs) for anti-HBV therapy, the incidence of HBV-related HCC has declined. However, HBV-HCC still occurs in some patients, with HBV integration playing a crucial role in this process.[9] Increasing research has focused on HBV integration events, revealing that integration of HBV genes with host genes is not a random occurrence;[10,11] rather, HBV gene fragments have a propensity to integrate into functional regions of the host genome, such as promoter regions and Cytosine-phosphate-Guanine (CpG) islands, thereby affecting host gene regulation and expression.[12,13] Research indicates that HBV integration can occur early after the host is infected and is detectable at all stages of chronic infection.[14] The frequent detection of HBV-host integration events suggest that this virus has an evolutionary advantage,[15] leading to biological effects throughout prolonged infection. For example, HBV S gene integration segments found early in infection can have

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long-term impacts on the host, independently producing surface antigens.[16] Even after antiviral treatment, these segments still contribute to the development of liver cancer.[13] Despite extensive research on HBV integration, its exact mechanisms remain unknown. Additionally, most studies are cross-sectional and only investigate integration characteristics at the time of HBV-HCC occurrence, neglecting long-term factors in the transition from chronic HBV infection to HCC development. These factors include the effects of long-term antiviral treatment, patient background in terms of liver disease, family history, and treatment compliance. Considering these factors, it is important to explore the pattern of HBV integration and its potential impact on patients' clinical characteristics, as there is relatively limited research in this area. Therefore, this study aims to investigate the pattern and characteristics of HBV integration in chronic HBV-infected patients who develop HBV-HCC despite receiving long-term antiviral treatment. Through long-term follow-up and GWAS, we seek to examine the HBV integration in liver cells of patients who eventually develop HBV-HCC. The primary objectives of this study are to explore the pattern and characteristics of HBV integration in this specific context, analyze its impact on patients' clinical indicators, and investigate the possible mechanisms underlying the observed clinical differences at the chromosome and genomic levels. This research will provide insights into whether HBV integration leads to abnormalities in human chromosome structure and genome sequences.

Patients and Methods

Study Patients

The study population consisted of a cohort of chronic HBV-infected patients who were followed for a long-term period. All patients were monitored from the beginning and received extended NUCs antiviral therapy based on standard treatment guidelines. The inclusion criteria for the study population were as follows: (i) Positive Hepatitis B surface antigen (HBsAg) at baseline; (ii) Long-term NUCs antiviral therapy; (iii) Detection of liver cancer through imaging examination, subsequent tumor resection, and histopathological confirmation of HCC; (iv) Continuous long-term follow-up. Conversely, the exclusion criteria comprised: (i) Co-infection with other viruses; (ii) No initiation or cessation of NUCs antiviral treatment; (iii) No occurrence of liver cancer; (iv) Loss to follow-up.

Study Design

The study utilized human specimens obtained from patients and was approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University (approval number: 2022−KT−E−142). In order to maintain ethical standards, the experiments were carried out following the Declaration of Helsinki guidelines. All patients involved in the study provided written informed consent.

This is a prospective study. Throughout the study, a comprehensive monitoring of various clinical parameters was conducted on all participants. In cases where liver cancer developed in the subjects, serum levels of alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) were measured. Tumor tissues obtained from surgical resection were collected for pathological examination and stored at −80°C. Subsequently, the frozen tissues were sent to Chi-Biotech Co., Ltd. (Shenzhen, PR China) for GWAS analysis and investigation of HBV integration status. Based on the test results, the study subjects were classified into two groups: the Int group and the N-int group. Quantitative analysis of clinical indicators, including HBV DNA, HBsAg, AFP, and DCP, was performed by the Clinical Laboratory Department at The First Affiliated Hospital of Guangxi Medical University. The Pathology Department of the same hospital conducted the pathological examination. This study aimed to investigate the patterns and characteristics of HBV integration in tumor tissues of patients who had developed HBV-HCC after long-term antiviral therapy. The differences in clinical parameters between the Int and N-int groups were compared to further elucidate the impact of HBV integration on human chromosome structure and genomic DNA. For a detailed description of the procedures involved in the study, please refer to Figure 1.

Whole-Genome Extraction, Library Preparation, and Sequencing

Tumor tissue DNA was extracted using the magnetic bead method. The purity of the DNA was assessed using the NanoPhotometer® spectrophotometer (IMPLEN, CA, USA), while the DNA concentration was measured using the

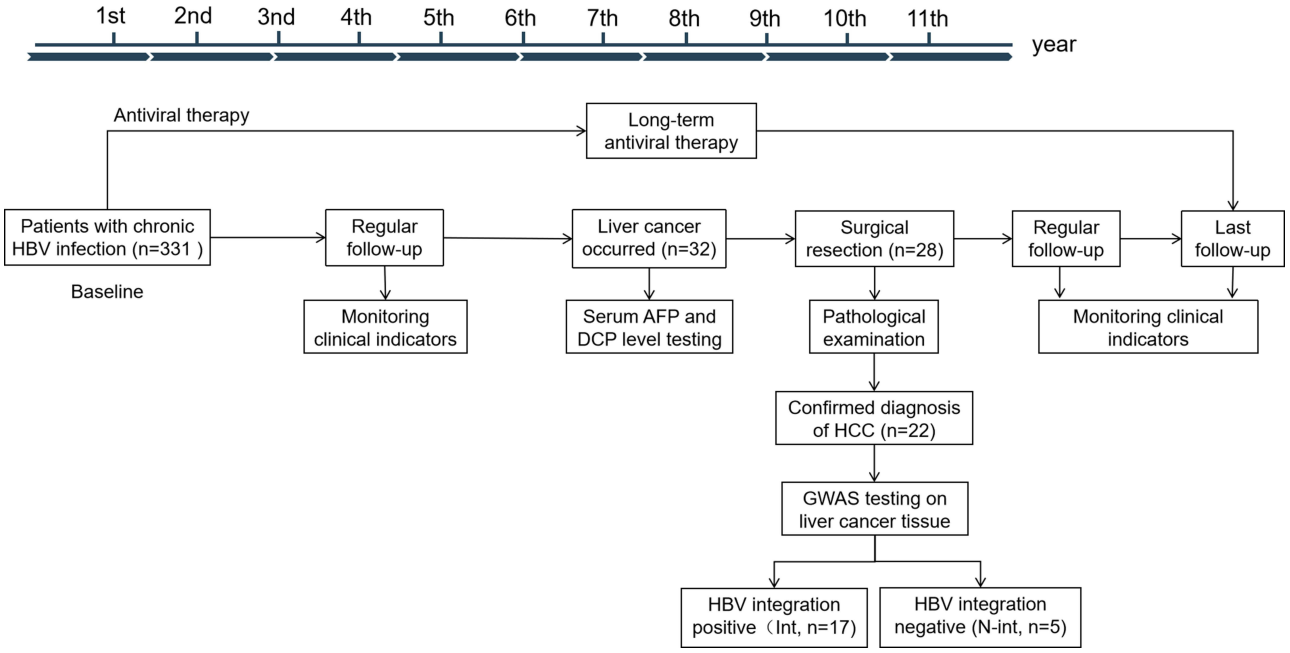

Figure 1 Research flowchart.
Abbreviations: HBV, hepatitis B virus; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; HCC, hepatocellular carcinoma; GWAS, genome-wide association study; Int, integration; N-int, none-integration.

Qubit® 3.0 Fluorometer (Life Technologies, CA, USA). A qualified sample with 0.5μg gDNA was selected as the template. Following the instructions provided in the Annoroad® Universal DNA Fragmentase kit V2.0 (Annoroad, Beijing, PR China), the qualified template was treated with reagents such as 10×FD and Fragmentase for DNA fragmentation. Subsequently, the Annoroad® Universal DNA Library Prep Kit V2.0 (Annoroad, Beijing, PR China) was used for end repair and adapter ligation. Magnetic beads with different ratios were utilized to select DNA fragments, and the desired fragments were enriched through PCR amplification, completing the library preparation process. After library construction, preliminary quantification was performed using the Qubit 3.0, and the library was diluted to a concentration of 1 ng/μL. The insert size of the library was assessed using the Agilent 2100 system. Once the insert size met the expected criteria, quantitative PCR was conducted using the Bio-RAD CFX 96 fluorescence quantitative PCR instrument (Bio-Rad Laboratories, CA, USA) and the Bio-RAD KIT iQ SYBR GRN (Bio-Rad Laboratories, CA, USA) to accurately determine the effective concentration of the library (library effective concentration > 10 nM), ensuring the quality of the library. The NovaSeq 6000 S4 Reagent kit V1.5 (Illumina, CA, USA) was used for cluster generation and sequencing on the NovaSeq 6000 S4 platform (Illumina, CA, USA). Paired-end sequencing was performed, generating 150 bp reads.

Detection of HBV Integration Sites

The adapter sequences were removed from the raw sequencing data using cutadapt software. The filtered reads were then aligned to both the human genome (hg19) and the HBV reference genome (NC_003977.2) using the bwa software. Duplicate sequences were removed using the picard tools. Finally, the seeksv software was utilized to identify the integration sites of the HBV virus. Determining the HBV integration sites involved the identification of split reads, where the two ends of a read mapped to different chromosomes. The breakpoint between the two chromosomes indicated the integration site.

Statistical Analysis

Data analysis was conducted using SPSS 27.0 statistical software (IBM, Armonk, NY, USA). Normally distributed continuous variables were represented as mean ± standard deviation (x ± s). For continuous variables that met the

assumptions of normal distribution and homogeneity of variances, independent samples *t*-test was used for between-group comparisons, while Welch's *t*-test was used when the assumption of equal variances was violated. Between-group comparisons were performed using one-way analysis of variance (ANOVA) and non-parametric tests for k independent samples. For repeated measures data, repeated measures analysis of variance was employed for comparisons. Correlation analysis between two variables was conducted using Pearson correlation analysis.

Results

Annotation and Visualization of HBV Integration Patterns in HBV-HCC After Prolonged Antiviral Therapy

In the analysis of 17 subjects with detected HBV integration, a total of 107 breakpoints were identified. By marking the location of the breakpoints on the human chromosomes, it was found that HBV gene fragment insertions were present on all chromosomes except chromosomes 6, 13, 20, and 22 (Figure 2A). Furthermore, the distribution of human genomic elements at the integration sites indicated a higher likelihood of HBV integration occurring in introns of human genes, followed by intergenic regions (Figure 2B). Gene ontology (GO) enrichment analysis of all interrupted genes at the HBV integration sites revealed their significant involvement in biological processes such as DNA-templated DNA replication, regulation of platelet activation, cellular response to dopamine, and regulation of Wnt signaling pathway ($p<0.05$, Figure 2C), which are important for maintaining genome stability and tumor development.

Additionally, the distribution of HBV integration breakpoints within the complete HBV genome revealed that the inserted HBV gene fragments were predominantly functional elements, with the most common insertions occurring in the Direct Repeat 1 (DR1, nt 1826-nt 1836) and the Basal Core Promoter (BCP, nt 1742-nt 1849) regions. Other commonly observed insertions included Enhancer 1 (Enh1, nt 1685-nt 1773), HBx gene promoter (xp, nt 1235-nt 1374), Enhancer 2 (Enh2, nt 1685-nt 1773), Direct Repeat 2 (DR2, nt 1560-nt 1570), Surface Promoter 1 (SP1, nt 2219-nt 2780), and Surface Promoter 2 (SP2, nt 2809-nt 3152) (Figure 2D). These functional elements primarily participate in HBV replication, transcription, and integration into the human genome. It was observed that HBV gene fragments had a preference for inserting near the chromosome termini and centromeres in the host. Within a 15Mb range of integration sites, 76.5% of patients had HBV gene fragment insertions near the chromosome termini, 64.7% had insertions near the centromeres, and even 35.3% of patients had insertions precisely at the centromeres (Figure 2E). Analyzing the distribution density of integrated HBV gene fragments near chromosome termini and centromeres in the complete HBV genome revealed that these gene fragments were mainly concentrated in the DR1, DR2, and BCP regions, with higher integration density at the chromosome termini compared to the centromeres regions (Figure 2F).

Furthermore, the correlation analysis between serum AFP and DCP levels and the number of reads at the integration breakpoints showed a strong correlation between serum AFP levels and breakpoint reads ($r=0.668$, $p=0.0034$, Figure 2G), while the correlation between serum DCP levels and breakpoint reads was not significant ($r=0.3830$, $p=0.1764$, Figure 2H).

General Characteristics of Subjects in Int and N-Int Groups

This study observed 331 patients with chronic HBV infection at baseline. All patients received long-term NUCs antiviral therapy. Eventually, 32 patients developed liver cancer, and out of these, 28 patients underwent surgical resection. Following pathological examination, 22 patients were diagnosed with HBV-HCC. Among them, 17 patients (77.3%) showed HBV integration in their liver cancer tissues and were categorized into the Int group, while the remaining patients were assigned to the N-int group. A comparison of the general characteristics of the Int and N-int groups is presented in Table 1.

Comparison of Clinical Indicators Between the Int and N-Int Groups

According to the guidelines for the prevention and treatment of chronic hepatitis B (2022 version),[17] the recommended treatment duration for NUCs antiviral therapy in patients with chronic HBV infection is at least 4 years. Therefore, to investigate whether there are differences in the changes of HBsAg levels between the Int and N-int groups after long-term antiviral treatment, we observed the dynamic changes in HBsAg levels in both groups starting from the 5th year. The results indicated that, regardless of

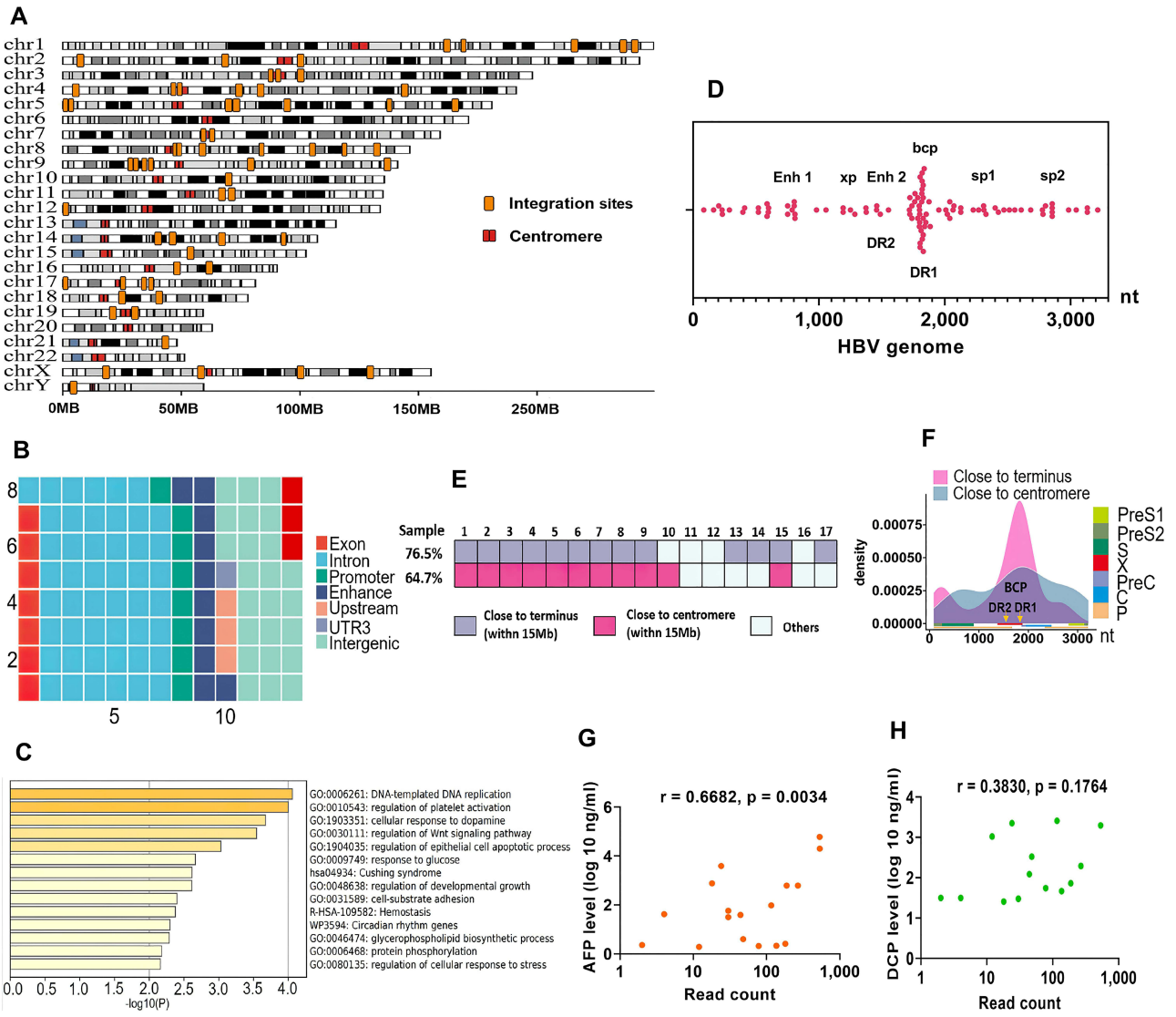

Figure 2 Annotation and visualization of HBV integration patterns. (**A**) The distribution of integration breakpoints on human chromosomes. (**B**) Wafer diagram depicting the distribution of human genomic elements at integration sites. (**C**) GO enrichment analysis on the interrupted genes at HBV integration sites. (**D**) The distribution of HBV breakpoints within the complete HBV genome. (**E**) Preferential proximity of HBV integration sites to chromosome terminus and centromere. (**F**) The density of HBV functional elements inserted near the termini and centromeres of human chromosomes. (**G** and **H**) Correlation analysis of AFP and DCP levels with read count at integration breakpoints. Correlation analysis between two variables was conducted using Pearson correlation analysis.

Abbreviations: Chr, chromosome; DR1, Direct Repeat 1; DR2, Direct Repeat 2; Enh1, Enhancer 1; Enh2, Enhancer 2; xp, HBx gene promoter; bcp, Basal core promoter; SP1, Surface Promoter 1; SP2, Surface Promoter 2; PreS1, hepatitis B virus pre-S1 region; PreS2, hepatitis B virus pre-S2 region; PreC, hepatitis B virus precore region.

the presence of HBV integration, both groups showed an overall decreasing trend in HBsAg levels after long-term antiviral treatment. However, there was a difference in the rate of decline, with the Int group exhibiting a slower decrease in HBsAg levels, which remained consistently higher than those in the N-int group from the 5th to the 9th year. At the last follow-up, the HBsAg levels in the Int group were significantly higher than those in the N-int group (564.4 vs 127.3 IU/mL, p=0.042, Figure 3A). In addition, there was a significant difference in the Barcelona Clinic Liver Cancer (BCLC) staging between the Int and N-int groups, with a higher proportion of patients in the Int group classified as advanced stages (x^2=5.0, p=0.026, Figure 3B).

The Impact of HBV Integration on Human Chromosome and Genomic DNA Sequences

The results of this study indicate a preference for HBV gene fragment integration into the termini regions and near the centromeres of human chromosomes. In the Int group, we observed that the integration of HBV into these two regions is

Table 1 The General Characteristics of Int and N-Int Groups

Factors	Int Group (N = 17)	N-int Group (N = 5)	*p*-value
Sex, n (%)			1.000
Male	14 (82.4)	4 (80)	
Female	3 (17.6)	1 (20)	
Age, years	48.4±12.5	52.8±10.3	0.532
Family history, n (%)			0.116
Positive	12 (70.6)	1 (20)	
Negative	5 (29.4)	4 (80)	
Baseline HBeAg, n (%)			0.135
Positive	7 (41.2)	0 (0)	
Negative	10 (58.8)	5 (100)	
Baseline HBV DNA level (log10 IU/mL)	4.9±1.1	4.5±1.1	0.452
Background liver disease, n (%)			1.000
CHB	6 (35.3)	1 (20)	
HBV-LC	11 (64.7)	4 (80)	
Treatment compliance, n (%)			1.000
Good	12 (70.6)	4 (80)	
Poor	5 (29.4)	1 (20)	
Switching to different medications, n (%)			1.000
Yes	12 (70.6)	4 (80)	
No	5 (29.4)	1 (20)	
Antiviral duration, years	11.0±3.0	10.4±2.1	0.686
HBsAg positivity to HCC development, years	17.5±6.4	24.8±5.0	0.030
Tumor size (cm)	3.4±1.8	1.8±2.2	0.023
AFP level at tumor onset (log 10 ng/mL)	1.9±1.5	1.8±1.7	0.967
DCP level at tumor onset (ng/mL)	2.2±0.8	2.7±1.7	0.400
Tumor recurrence, n (%)			1.000
Yes	8 (47.1)	3 (60)	
No	9 (52.9)	2 (40)	

Notes: Categorical variables were presented as n (%) and analyzed using the chi-square test. Continuous variables were expressed as mean ± SD and evaluated using the *t*-test. $p<0.05$ indicates a statistically significant difference.
Abbreviations: Int, integration; N-int, none-integration; HBeAg, Hepatitis B e antigen; CHB, chronic hepatitis B; HBV-LC, HBV-related liver cirrhosis; AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.

likely to lead to structural changes in human chromosomes. For instance, in sample 2, we identified two instances of HBV gene fragment integration at the centromere region of chromosome 3, specifically the HBx and HBp gene fragments. Within the range of 50–95 Mb on this chromosome, a segment of the genome was found to be deleted

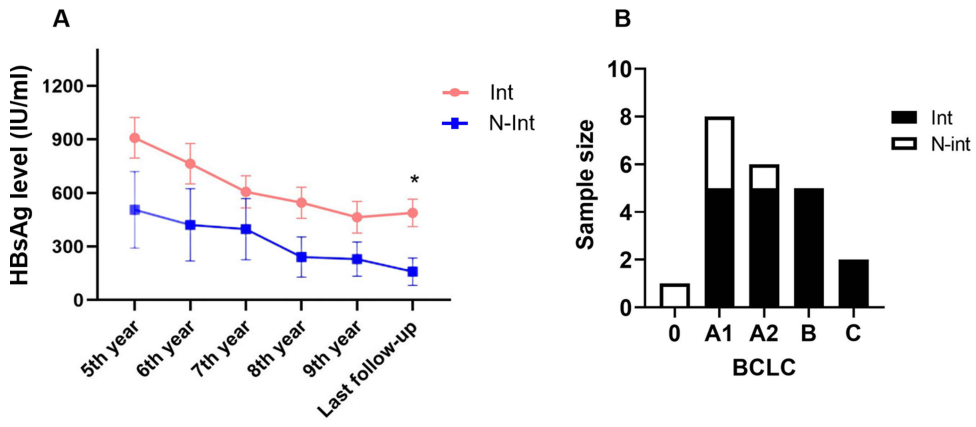

Figure 3 Comparison of clinical indicators between the Int and N-int groups. (**A**) Dynamic changes in HBsAg levels between the Int group and the N-int group. Comparison of group differences using repeated measures analysis of variance. (**B**) Comparison of BCLC staging between Int and N-int groups. *$p<0.05$.
Abbreviations: HBsAg, Hepatitis B surface antigen; BCLC, Barcelona Clinic Liver Cancer.

(Figure 4A). Further analysis revealed that this deleted segment of the DNA sequence underwent fragmentation and rearrangement with DNA sequences from chromosomes 7, 10, 15, and 22. Similarly, at the breakpoint of HBV integration, the cms1 ribosomal small subunit homolog (CMSS1) gene on chromosome 11 underwent rearrangement with the SH3 and multiple ankyrin repeat domains 2 (SHANK2) gene, resulting in the formation of a fusion gene called CMSS1-SHANK2. The detection rate of this fusion gene in the Int group was 52.9% (9/17) (Figure 4B). In contrast, no such phenomenon was observed in the N-int group, and no CMSS1-SHANK2 fusion gene was detected (Figure 4E). Additionally, near the termini regions of human chromosomes, similar phenomena were observed. For example, in sample 8, a segment of the genome was deleted in the range of 93–110 Mb at the terminus of chromosome 14, where integration of the HBc gene fragment was identified (Figure 4C). This deleted segment of the DNA sequence underwent rearrangement with DNA sequences from chromosomes 15 and 17, and the serpin family A member 5 (SERPINA5) gene near the integration breakpoint underwent rearrangement with intergenic sequences on chromosome Y, resulting in the formation of a fusion sequence called SERPINA5-Intergenic. The detection rate of this fusion sequence in the Int group was 70.6% (12/17) (Figure 4D). In contrast, no such phenomenon was observed in the N-int group (Figure 4F).

Furthermore, this study found that HBV integration may impact the genomic DNA sequences. In the Int group, it was observed that half of the patients had HBV gene fragments integrated into the exon region or promoter region of the Telomerase Reverse Transcriptase (TERT) gene. This may explain the significant increase in TERT gene copy numbers in the Int group compared to the N-int group (p=0.039, Figure 4G). Further analysis showed that the TERT gene SNV deletion number in the Int group was significantly higher than in the N-int group (p=0.019, Figure 4H), and the SNV substitution number was also significantly higher in the Int group compared to the non-integration group (p=0.016, Figure 4I). However, there was no significant difference in SNV insertion number between the two groups (p=0.178, Figure 4J). These results suggest that HBV integration may increase the occurrence rate of SNVs in certain key genes.

Discussion

HBV integration is closely associated with the development of HBV-HCC, and previous studies have extensively investigated the role and mechanisms of HBV integration in the formation of HBV-HCC.[15,18] However, most studies have focused solely on the HBV integration mechanism at the time of HBV-HCC occurrence, neglecting the process from chronic HBV infection to HBV-HCC development. For instance, few studies have examined whether patients have undergone long-term antiviral therapy or the disease background before the occurrence of HBV-HCC. In this study, we selected individuals with chronic HBV infection who developed HBV-HCC despite receiving long-term antiviral therapy and conducted a comprehensive investigation into the patterns and characteristics of HBV integration in the context of HBV-HCC development through long-term follow-up.

Consistent with previous findings, this study also revealed that HBV integration is common in HBV-HCC. However, not all subjects exhibited evidence of HBV integration, indicating that the occurrence of HCC may not solely be attributed to HBV integration. Unlike previous reports of a high number of integration breakpoints detected in HBV-HCC patients,[19,20] this study identified only 107 integration breakpoints in 17 patients with HBV integration. Additionally, irrespective of the presence of HBV integration, HBsAg levels exhibited a gradual decline over the course of nearly 11 years of antiviral therapy in both the Int group and N-int group. Nevertheless, the presence of HBV integration was associated with a slower decrease in HBsAg levels, and this difference progressively increased over time (Figure 3A). These findings suggest that long-term antiviral therapy may reduce the occurrence of HBV integration events, including the number of HBs gene integrations capable of independently producing HBsAg, thereby leading to a gradual decrease in HBsAg levels.[8,21]

Furthermore, substantial differences were observed in the number of HBV integration events among different patients, and these differences may have implications for tumor markers. This study demonstrated a positive correlation between AFP levels and the number of integration events, suggesting that the number of integration events may influence the measurement of AFP levels. This finding may provide an alternative explanation for the occurrence of false-negative AFP levels in some HCC patients during clinical diagnosis. Conversely, the correlation between DCP levels and integration events was not strong, indicating that integration events may not have a significant impact on DCP levels. This further supports the rationale for utilizing AFP and DCP in combination for clinical diagnosis of HCC.

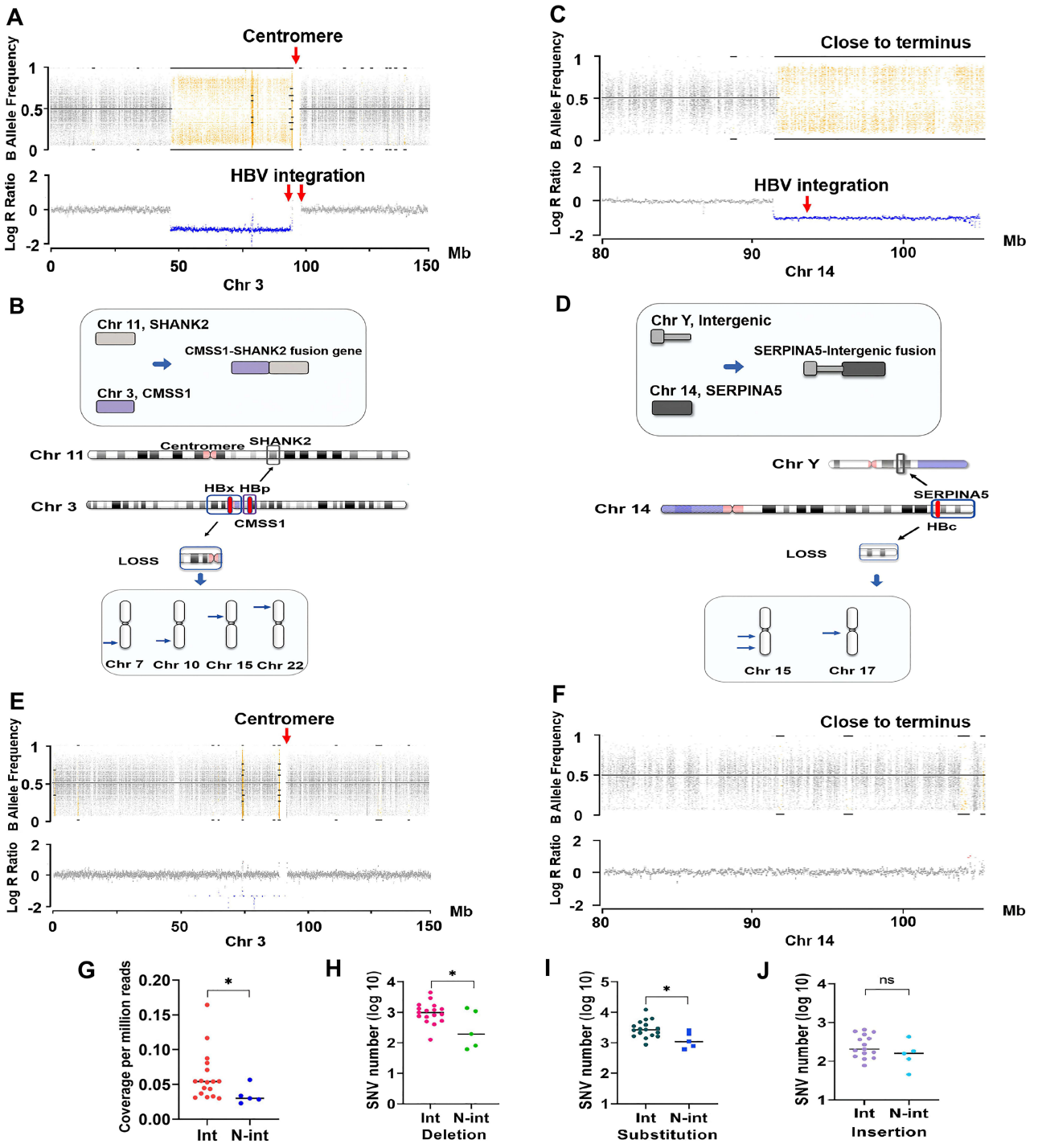

Figure 4 The impact of HBV integration on human chromosome and genomic DNA sequences. (**A** and **B**) Integration of HBx and HBp gene fragments near the centromere of chromosome 3 was observed in sample 2. B Allele Frequency and Log R Ratio analysis indicated that HBV integration may lead to a genomic deletion in this region. The deleted genomic sequences were found to be fused with DNA sequences from chromosomes 7, 10, 15, and 22. The CMSS1 gene at the integration breakpoint of chromosome 3 rearranged with the SHANK2 gene on chromosome 14 to form the CMSS1-SHANK2 fusion gene. (**C** and **D**) In sample 8, integration of the HBc gene fragment was observed at the termini of chromosome 14, also leading to the deletion of a genomic segment in this region. The deleted genomic sequences were found to be fused with DNA sequences from chromosomes 15 and 17. The SERPINA5 gene at the integration breakpoint of chromosome 14 was fused with the intergenic region of the Y chromosome to form the SERPINA5-Intergenic fusion sequence. (**E** and **F**) B Allele Frequency and Log R Ratio analysis revealed no genomic deletions on chromosomes 3 and 14 in the N-int group. (**G–J**) Comparison of TERT gene copy number and the number of TERT gene deletion, substitution, and insertion SNVs were performed between the Int and N-int groups. Loss of heterozygosity (LOH) regions are indicated in yellow in panels A and C, while blue represents genomic deletions. *$p<0.05$.

Abbreviations: Ns, not significant; CMSS1, cms1 ribosomal small subunit homolog; SHANK2, SH3 and multiple ankyrin repeat domains 2; SERPINA5, serpin family A member 5; HBx, hepatitis B virus X protein gene; HBp, hepatitis B virus polymerase gene; HBc, hepatitis B virus core gene; SNV, single nucleotide variation.

When comparing the general characteristics between the Int and N-int groups, distinct effects of the presence or absence of HBV integration on specific clinical indicators in HBV-HCC patients were observed. For instance, under the condition of receiving a similar duration of antiviral therapy, the time from detection of HBsAg positivity to the development of HCC was shorter in the Int group than in the N-int group (Table 1), suggesting that HBV integration may contribute to the acceleration of HBV-HCC development to some extent. Moreover, a significant disparity in tumor diameter was observed between the Int and N-int groups at the time of liver cancer diagnosis. Most patients in the Int group exhibited tumor diameters exceeding 3 cm, indicative of medium or large liver cancer, while the majority of patients in the N-int group had tumor diameters less than 3 cm, signifying small liver cancer (Table 1). This divergence in tumor growth and proliferation may partially elucidate the significant discrepancy in BCLC staging between the two groups (Figure 3B).The research findings mentioned above offer a new perspective on the diagnosis and treatment of HBV-HCC patients. For instance, we should focus on integration events occurring in HBV-infected individuals. Additionally, eliminating HBV integration may reveal new therapeutic approaches for these patients.

To investigate the potential mechanisms underlying the differences in clinical indicators between the Int and N-int groups caused by HBV integration, we conducted chromosomal and genomic analyses. Our findings revealed that HBV integration events primarily occur near the termini and the centromeres regions of human chromosomes, as well as within intronic and intergenic regions of DNA sequences.[22] Chromosomal abnormalities and changes in stability are prominent outcomes of high-frequency HBV integration at these regions.[23] The insertion of HBV gene fragments frequently causes the partial loss of adjacent genomic regions, resulting in segmentations and subsequent gene rearrangements with other chromosomes, leading to the formation of fusion genes or fusion sequences (Figure 4A–D). In contrast, these phenomena were not observed in patients from the N-int group (Figure 4E and F). Notably, this study identified a significantly high occurrence of CMSS1-SHANK2 fusion genes and SERPINA5-Intergenic fusion sequences in HBV-HCC patients. Previous studies have demonstrated the role of CMSS1-FLT1 fusion genes in the development of acute myeloid leukemia,[24] and the findings of SHANK2-FGFR1 fusion genes in colorectal cancer have been extensively researched.[25] Additionally, SHANK2 itself has been implicated in tumor formation.[26] Furthermore, the SERPINA5 gene has been associated with the promotion of gastric cancer cell proliferation.[27] Additionally, this gene also plays a significant role in the development of hepatocellular carcinoma.[28] The detection of CMSS1-SHANK2 fusion genes and SERPINA5-Intergenic fusion sequences in HBV-HCC for the first time in this study, coupled with their high detection rate in HBV-HCC patients, suggests their potential as novel detection sites or biomarkers for HBV-HCC.

Apart from its influence on chromosomal structure, HBV integration also impacts the sequence of the human genome.[29] Integrated HBV fragments often contain functional regulatory elements,[14] contributing to the abnormal expression of human genes at the insertion site or an increased rate of SNVs (Figure 4G–J). These observations highlight the synergistic interaction between the human genome and HBV in mediating the biological effects resulting from HBV integration events.[30] Furthermore, the presence of HBV DR1, DR2, and BCP functional elements in high density near the termini and the centromeres regions of human chromosomes further supports this notion (Figure 2F).

This study has certain limitations. Firstly, the long duration of follow-up and the overall low incidence of liver cancer have resulted in a small sample size, potentially introducing biases into the study results. Secondly, additional experimental validation is necessary for some of the findings obtained in this study.

Conclusion

In summary, this research examined the patterns and characteristics of HBV integration in tumor tissues of patients with HBV-HCC who were undergoing long-term antiviral therapy for chronic HBV infection. The results of the study suggest that HBV integration is relatively infrequent following long-term antiviral therapy, but it can still have an impact on the clinical indicators of patients. HBV integration tends to occur near the termini and centromeres of human chromosomes, affecting the structure and stability of chromosomes, and leading to gene rearrangement events. Additionally, HBV integration may cause abnormalities in the sequence of human genomic DNA and a notable increase in SNVs. The integration elements of HBV often contain functional regulatory elements, indicating the involvement of both HBV and the human genome in HBV integration events. The identification of novel fusion genes may offer new opportunities for early detection of HBV-HCC.

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Disclosure

The authors report no conflicts of interest in this work.

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