HBV integrated genomic characterization revealed hepatocyte genomic alterations in HBV-related hepatocellular carcinomas

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Abstract. Hepatocellular carcinoma (HCC) is one of the most lethal malignancies that is closely associated with the Hepatitis B virus (HBV). HBV integration into host genomes can induce instability and the aberrant expression of human genomic DNA. To directly assess HBV integration breakpoints at whole genome level, four small sequencing libraries were constructed and the HBV integration profiles of four patients with HCC were characterized. In total, the current study identified 11,800,974, 11,216,998, 11,026,546 and 11,607,842 clean reads for patients 1-3 and 4, respectively, of which 92.82, 95.95, 97.21 and 97.29% were properly aligned to the hybrid reference genome. In addition, 220 HBV integration events were detected from the tumor tissues of four patients with HCC and an average of 55 breakpoints per sample was calculated. The results indicated that HBV integration events may be implicated in HCC physiologies and diseases. The results acquired may also provide insight into the pathogenesis of HCC, which may be valuable for future HCC therapy.

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Introduction

Hepatocellular carcinoma (HCC), one of the highest prevalence of liver cancer, caused by hepatitis B virus (HBV), and more than 350 million people were affected by HBV worldwide (1,2). All over the world, majority of HBV-related deaths were closely associated with HCC for each year, which indicates the third leading cause of cancer deaths (2,3). As we known, the HBV was a major etiologic agent for HCC, and prevalent in China, Southeast Asia and sub-Saharan Africa (4). Notably, an increasing number of studies indicated that HBV was implicated in tumorigenesis, of which the main mechanism as follows (3-7): i) Expression of viral proteins, in particular, from HBV gene X (HBx), to modulate cell proliferation and viability, ii) accumulation of genetic damage due to hepatic inflammation mediated by virus-specific T cells, and iii) it is intriguing that integration of HBV DNA into the host genome to alter the function of endogenous genes or induce chromosomal instability.

There are increasing evidences that the events of the HBV integration occurred in the HCC, and affected function of HCC genome. Some researchers found that HBV DNA integration distributed different chromosomal sites in the host genome (8), and they could trigger chromosomal changes, genomic instability, or changes in the expression of human genes (9). It was reported that HBV integration events are involved in chromosome fragile sites or repetitive sequences and are usually followed by local rearrangement, all of which relate to higher genomic instability (10). HBV insertion was found to target the retinoic acid receptor-β (RARB) gene or the human cyclin A2 (CCNA2) and to generate chimeric oncogenic proteins (11). Until now, all reports involving HBV DNA integration implied that integration plays a role in the transformation (12-14). HBV integration into the host cell genome has also been reported, which resulted in gene mutations, insertions, deletions or rearrangements of the host genome (15-17). Recently, a great number of insertion sites were identified by next-generation sequencing (4,18,19). Interestingly, telomerase reverse transcriptase (TERT) and mixed-lineage leukemia 4 (MLL4) gene were frequently targeted by HBV in HCC tissue, and the latter may play a major role in HCC carcinogenesis (20). Since firstly discovered

at the TERT gene, HBV integration breakpoints have been widely reported in some gene targets, such as the fatty acyl CoA reductase 2 (FAR2), inositol triphosphate receptor type 1 (ITPR1,IP3R1), Interleukin-1-receptor-associated kinase 2 (IRAK2), mitogen associated protein kinase 1 (MAPK1), mixed-lineage leukemia 2 (MLL2) and MLL4 genes (2,21,22). Although HBV integration event has been reported in the HCC, and its mechanism is not clear. Therefore, systematic analysis of HBV integration targets could elucidate HCC physiologies and diseases development processes, and predict novel therapeutic targets. Here, to directly detect HBV integration breakpoints at whole genome level, we constructed four small sequencing libraries and characterized the HBV integration profiles from four patients with HCC. Therefore, our research revealed HBV integration events in the HCC, and acquired insight into the pathogenesis of HCC.

Materials and methods

Human samples and DNA extractions. Four tumor samples were collected from the patients who underwent curative primary hepatectomy or liver transplantation in the Guilin No. 924 Hospital. They were have precisely diagnosed with HCC and associated with HBV infection in the department of pathology, and the hepatitis B surface antigen (HBsAg) was positively expressed and HBV-DNA quantification was greater than 10^3 copies/ml (Fig. 1A and B and Table I). Subsequently, total DNA was extracted from the tumor samples using QIAamp DNA Micro kit (Qiagen Ltd.) according to the manufacturer's methods. Informed consent was obtained from the participant donors, and the protocol for the research project has been approved by a Ethics Committee of the Guilin No. 924 Hospital accord with China's Guidelines.

Hybridized Libraries construction and sequencing. DNA purification and library preparation were conducted as previously reported methods (2). Brief, integrity and quality of total DNA were evaluated by the Qubit Fluorometer and agarose gel electrophoresis (Fig. 1). Next, the genomic DNA were fragmented randomly by a Bioruptor Pico (Diagenode, B01060001) into the target DNA fragments (170 bp) from 3 μg of total DNA. And then, the target fragments were subjected to perform DNA end repair, and added a single 'A' nucleotide in the 3' ends of the target DNA fragments. Subsequently, PCR were performed after adapters ligating, size-selection and tailed random primers addition to obtain sufficient amplification products for libraries construction. For these constructed libraries, we used the virus probe to hybridize, and enriched the target DNA fragments. The hybridized target DNA fragments were eluted using AW2 Buffer in Elution column (QIAamp minElute Column) And then, PCR were performed to obtain sufficient amplification products for the hybridized libraries construction. PCR products were collected and preceded to 101 cycle's paired-end index sequencing in the Illumina HiSeq 2,000 sequencer according to manufacturer's instructions (Illumina Inc.).

Hybrid reference genome construction and alignment analysis. The advanced analysis begins with raw data generated from the Illumina platform. The sequence tags with adapter ligation or low quality or Ns and low base quality were filtered out to obtain clean tags, which were subjected to further analyze. Next, we combined the human reference genome (hg19) and the HBV genome (NC_003977.1) together to build a hybrid reference genome for alignment analysis. The clean tags were aligned to the hybrid reference genome by Burrows-Wheeler Aligner (BWA) (23), and the alignment results were saved in BAM format files. These files were further preprocessed to be the final BAM files for the HBV integration detection, such as sorting according to the alignment position, marking duplicate reads caused by PCR.

SNPs and InDels detection and annotation. Further bioinformatic analysis for the final BAM files, single Nucleotide Polymorphisms (SNPs) are detected by GATK (24). The unique genotypes were identified for each individual, which has the highest probability at a given locus, the consensus sequence tags were collected and saved as CNS format. And then, the high confidence SNP datasets were acquired by filtering the consensus sequence tags. In addition, we detect the small Insertion and Deletion (InDels) using pair-end reads for gap alignment.

HBV integration detection. We detected the HBV integrations by seeksv, and the seeksv was an in-house tool. The HBV integration positions were filtered, because of the sequences with a sum of junction read number and abnormal read pair number smaller than 2. After integration positions identification, the distribution of the HBV integrations was analyzed, which could evaluate the numbers of integrations.

Results

Analysis of sequencing data from four tumor samples. Integrated genomic DNA was captured from tumor tissues of four patients with HCC and sequenced by Illumina HiSeq 2,000. Before doing any further analysis, quality control is essential to detect whether the data is qualified. As shown in Fig. 2, the sequencing data was good, which satisfied the subsequent advanced analysis. These raw reads with adapters, low quality and unknown bases were removed. The remaining data were called as clean read data, which were submitted for further bioinformatic analysis. In total, an average of 11,413,090 raw reads were obtained for each sample. A Phred quality score is a measure that was used to evaluate the quality for sequencing data. Phred quality scores sQ were defined as the sequencing data quality from tumor tissues of four patients, and the *E* indicated the sequencing error rate. They had a relationship as follows: sQ=-10log$_{10}$*E*. It's worth noting that the GC content accounted for 43.68-44.64% when the Phred score was >30.

Further statistical analysis for clean reads, we identified 11,800,974, 11,216,998, 11,026,546 and 11,607,842 clean reads for Patient 1-3 and 4, of which 92.82, 95.95, 97.21 and 97.29%, respectively, were properly aligned to the hybrid reference genome. The average sequencing depths were 0.07, 0.08, 0.05 and 0.07-fold, and 5.47, 6.17, 3.63 and 4.93 of the hybrid reference genome were covered by the clean reads, respectively. Coverage at least 20-fold were 0.01% for four tumor tissues (Table II).

HBV integration analysis for four tumor tissues. In order to explore the HBV integration events in the HCC patients, we used the seeksv to detect the HBV integration sites, and

Table I. Clinical and biochemical characteristics of the four patients prior to whole-genome sequencing.

ID	Age (years)	Sex	HBsAg	Anti-HBs	Anti-HBc	AFP	HBV (copies/ml)
1	38	Male	+	-	+	+	9,337
2	52	Male	+	-	+	+	6,060
3	42	Male	+	-	+	+	3,504
4	50	Male	+	-	+	+	2,139

HBsAg, hepatitis B surface antigen; Anti-HBs, antibody to hepatitis B surface antigen; Anti-HBc, antibody to hepatitis B core antigen; AFP, α-fetoprotein; HBV, hepatitis B virus.
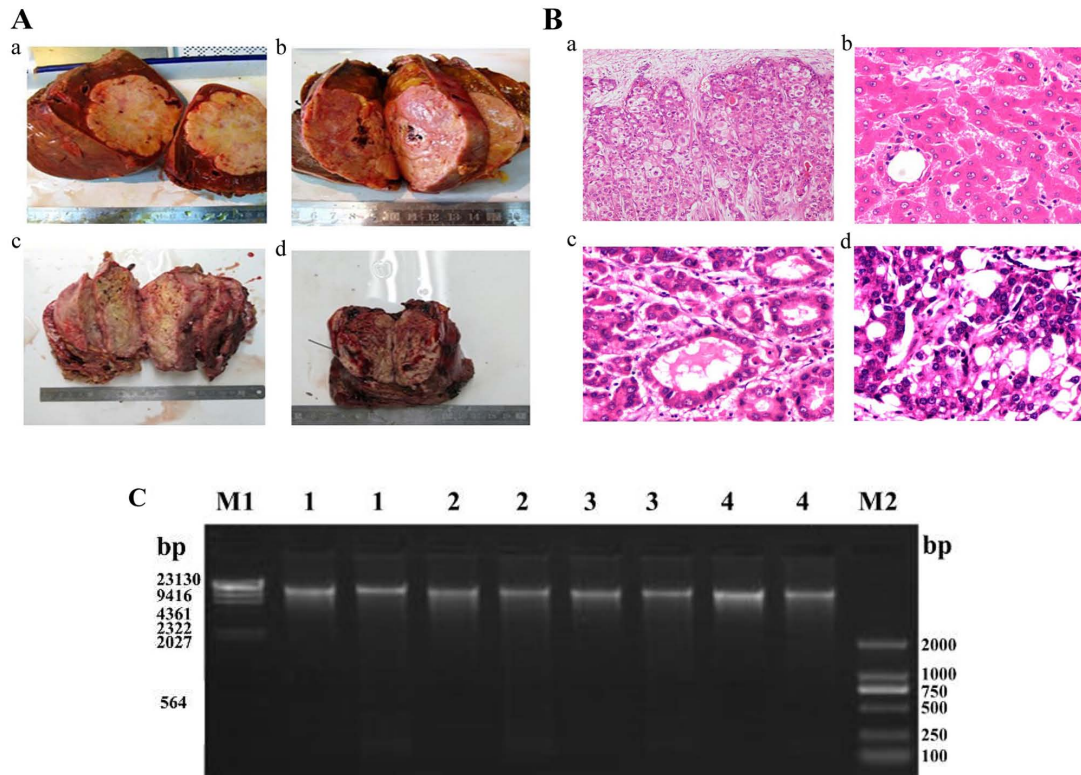

Figure 1. Clinical characteristics of the four patients enrolled for deep sequencing. (A) Excised tumors of the four patients with HCC included in the present study (Aa, patient 1; Ab, patient 2; Ac, patient 3; Ad, patient 4). (B) Pathological images of tumor tissues from the four patients with HCC (magnification, x20; Ba, patient 1; Bb, patient 2; Bc, patient 3; Bd, patient 4). (C) Integrity analysis of total DNA using preparative agarose gel electrophoresis. HCC, hepatocellular carcinoma.

conducted the annotation and classification by ANNOVAR. In total, the 220 HBV integration events were detected from the tumor tissues of four HCC patients, and an average of 55 breakpoints for each sample. The integration breakpoints distributions for four samples were 143, 40, 25 and 12, respectively (Fig. 3), which evaluated the numbers of integrations located in different gene regions. Our results indicated that the HBV integration breakpoints had a preference for chromosomes 2, 3 and 5 (Table III). Majority of HBV breakpoints in HCC were found near coding genes (116 of 220 breakpoints). Eight of the 220 HBV breakpoints were located in known coding genes, such as ITGA9, FAM19A4, TTBK1, POT1, FLNC, OR51V1, HSPA4, ITGA4, and these breakpoints were significantly over-represented in exon and promoter (defined as 0 to -2 kb relative to the transcriptional start site) regions. In contrast, 85 of 220 HBV breakpoints were mainly located in introns. In addition, we identified 688 potential mutations of SNPs four samples by aligning to the hybrid reference genome. Majority of SNPs (666/688) were somatic single-base mutations, while the minority (22/688) occured small insertions and deletions (InDels), which were detected by pair-end reads for gap alignment (Table SI).

Discussion

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies, and it's development was a multifactorial process because of several direct and indirect mechanisms (25). HBV DNA integration events were frequently detected in the HCC patients, which was observed more usually in the tumors than in adjacent liver tissues, and was associated with patient's survival (4,21). Exactly as our clinical examination results for four HCC patients, the hepatitis B surface antigen (HBsAg) was positively expressed, and HBV DNA integration was

Table II. Quality control results of alignment data.

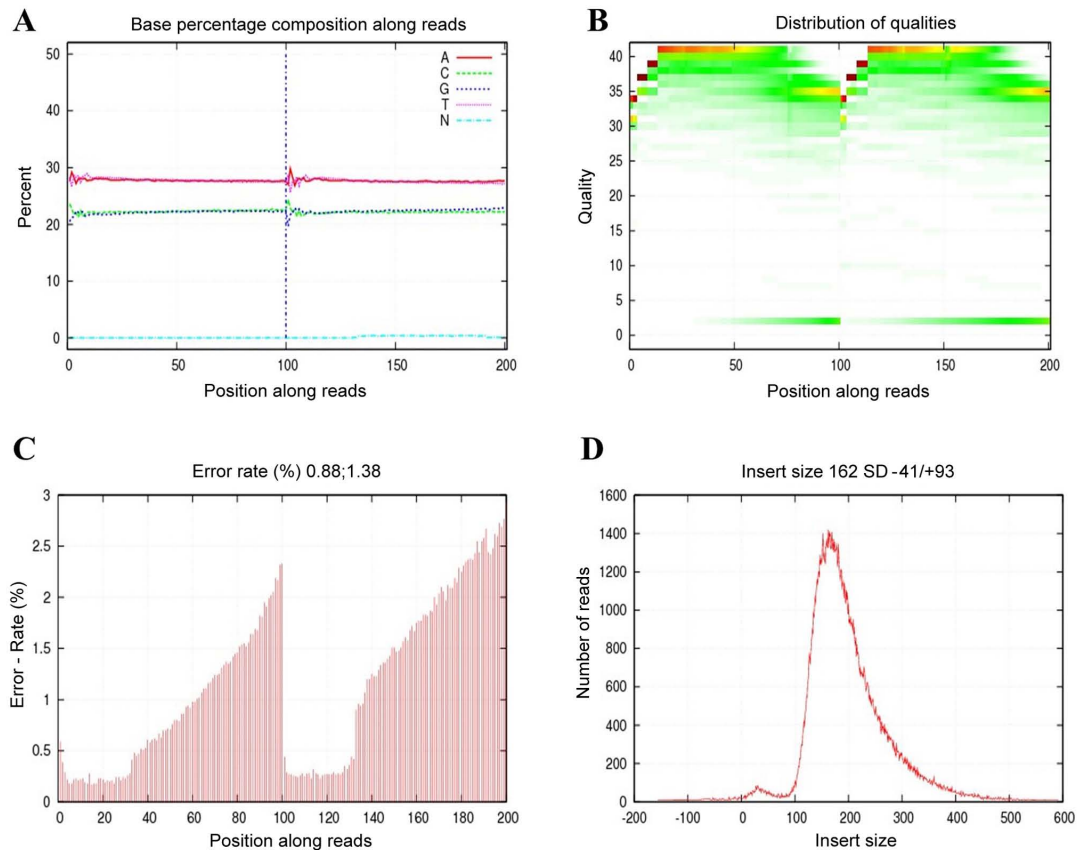| Categories	Patient 1	Patient 2	Patient 3	Patient 4
Clean reads	11,800,974	11,216,998	11,026,546	11,607,842
Clean bases (bp)	1,180,097,400	1,121,699,800	1,102,654,600	1,160,784,200
Mapped reads	10,953,113	10,763,243	10,718,360	11,293,616
Mapped bases (bp)	1,075,438,128	1,058,305,701	1,049,564,676	1,108,297,233
Mapping rate (%)	92.82	95.95	97.21	97.29
Uniq reads	10,295,380	10,118,513	10,040,852	10,627,022
Uniq bases (bp)	1,012,882,982	996,468,729	985,514,390	1,045,019,717
Unique rate (%)	94.00	94.01	93.68	94.10
Duplicate reads	8,692,617	8,282,575	9,152,300	9,265,976
Duplicate rate (%)	79.36	76.95	85.39	82.05
Mismatch bases (bp)	5,924,651	4,942,501	5,471,027	4,874,040
Mismatch rate (%)	0.55	0.47	0.52	0.44
Average sequencing depth	0.07	0.08	0.05	0.07
Coverage (%)	5.47	6.17	3.63	4.93
Coverage at least 4X (%)	0.05	0.05	0.05	0.05
Coverage at least 10X (%)	0.02	0.02	0.02	0.02
Coverage at least 20X (%)	0.01	0.01	0.01	0.01

Figure 2. Quality analysis of the sequencing data obtained from the four tumor samples. (A) Balanced base composition of raw reads. (B) High quality distribution of bases along reads. (C) The distribution of error rate along reads. (D) The distribution insert size of DNA fragment.

frequently detected in the most HCC patients (26). Linghao Zhao and his colleagues identified 4,225 HBV integration events in tumor and adjacent non-tumor samples from 426 patients with HCC, and found that they preferred rare fragile sites and functional genomic regions, such as CpG islands (27). In addition, some researchers observed massive genomic perturbations near viral integration sites, such as direct gene disruption, viral promoter-driven human

Table III. Distribution of the targets in chromosomes.

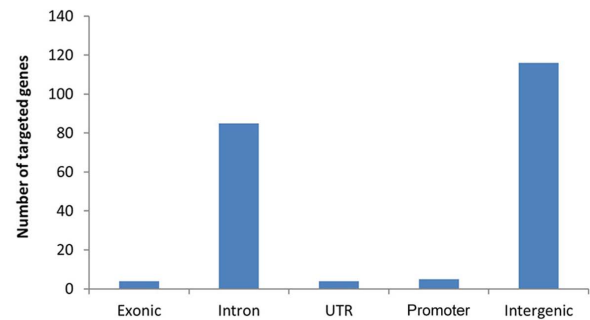| chr	Patient 1	Patient 2	Patient 3	Patient 4
chr1	9	3	1	0
chr2	10	4	6	8
chr3	11	3	6	0
chr4	4	0	2	0
chr5	10	1	8	0
chr6	5	1	0	2
chr7	6	0	3	0
chr8	3	1	0	1
chr9	6	2	0	0
chr10	6	3	0	0
chr11	7	0	1	0
chr12	3	0	2	0
chr13	10	0	1	0
chr14	8	0	0	0
chr15	8	2	1	0
chr16	4	2	0	0
chr17	6	0	3	0
chr18	3	2	0	0
chr19	3	0	0	1
chr20	8	0	2	0
chr21	0	0	0	0
chr22	5	0	0	0
chrX	2	0	0	0

Chr, chromosome.

Figure 3. HBV integration breakpoint analysis for four tumor samples. Data are presented as the distribution of HBV integration breakpoints in different gene regions.

transcription, viral-human transcript fusion and DNA copy number alteration (28).

Traditionally, majority of the HBV integration events were detected by PCR-based methods, such as Alu-HBV PCR, and preferred the Alu regions (2). As a result, the HBV integration that located in the Alu regions can be frequently detected (2). Several studies have shown that the whole-genome sequencing combined with HBV DNA capture for HBV DNA integration events detection was feasible and effective, with considerable savings in time and cost, and could help improve the early diagnosis of hepatocellular carcinomas, and thus the survival rate. (4,21,27). In our study, we selected the whole-genome sequencing combined with HBV DNA capture to identify the HBV integration breakpoints from four tumor samples, which learns about the biological characteristics of HBV integrated into the human genome and provides some references for targeted therapy of HCC patients in the future.

Notably, we successfully identified the HBV integration breakpoints at the single base level, which can effectively validate confidence of HBV capture sequencing (2). Our data indicate that a large proportion of SNPs (666/688) were identified, which suggested the main tendency of HBV integration, and the InDels occasionally occurred in the HBV integration events. Subsequently, we analyzed the HBV integration breakpoints that distributed in distinct genomic elements, and found that majority of HBV integration breakpoints in the four

tumor samples located in the coding region (116 of 220 HBV integration breakpoints). Of the 220 HBV integration breakpoints, 9 were located in known coding genes that significantly over-represented in exon and promoter regions. In contrast, 85 of 220 HBV integration breakpoints were mainly located in the introns, which suggested the preference among the HBV integration breakpoints. It is intriguing that virus genes inserted into host genomic DNA in totally random ways in the previously studies (29). However, some recent studies indicated that virus genes integration events had preference among the different regions (4,9,19). Our HBV integration profiles also demonstrated that HBV integration breakpoints preferentially landed in transcription units and specific chromosomes. Ding et al (9) found the HBV integration preference located in chromosomes 11 and 17. However, a preference for chromosome 3 has been reported in chronic hepatitis tissues without HCC by Alu-PCR (21). Our results indicated that the HBV integration breakpoints had a preference for chromosomes 2, 3 and 5. There are some potential reasons for the preferences, including the great HCC heterogeneity, different subclasses of HCC, sub-genotypes with different integration capabilities, etc. Therefore, we need a large number of samples to verify the results of this study, further explore the function of these target genes in the pathological mechanism of HCC and the characteristics they are in the TCGA database, which will provide more references for our future to discussion of 'therapeutics in HCC patients. Additionally, future analysis for the potential relationship between HBV subtypes and their integration frequencies was needed in the future.

In summary, our results strengthened understanding that the HBV DNA integration events are implicated in HCC physiologies and diseases, and further demonstrated that the HBV insertional sequence capturing may be a useful tool to study the related human diseases.

References

1. Ringelhan M, Pfister D, O'Connor T, Pikarsky E and Heikenwalder M: The immunology of hepatocellular carcinoma. Nat Immunol 19: 222-232, 2018.
2. Li W, Zeng X, Lee NP, Liu X, Chen S, Guo B, Yi S, Zhuang X, Chen F, Wang G, *et al*: HIVID: An efficient method to detect HBV integration using low coverage sequencing. Genomics 102: 338-344, 2013.
3. Wu G, Ding H and Zeng C: Overview of HBV whole genome data in public repositories and the Chinese HBV reference sequences. Prog Nat Sci 18: 13-20, 2008.
4. Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, *et al*: Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. Nat Genet 44: 765-769, 2012.
5. Gehring AJ, Ho ZZ, Tan AT, Aung MO, Lee KH, Tan KC, Lim SG and Bertoletti A: Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma. Gastroenterology 137: 682-690, 2009.
6. Lau CC, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, *et al*: Viral-human chimeric transcript predisposes risk to liver cancer development and progression. Cancer Cell 25: 335-349, 2014.
7. Neuveut C, Wei Y and Buendia MA: Mechanisms of HBV-related hepatocarcinogenesis. J Hepatol 52: 594-604, 2010.
8. Tokino T and Matsubara K: Chromosomal sites for hepatitis B virus integration in human hepatocellular carcinoma. J Virol 65: 6761-6764, 1991.
9. Ding D, Lou X, Hua D, Yu W, Li L, Wang J, Gao F, Zhao N, Ren G, Li L and Lin B: Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. PLoS Genet 8: e1003065, 2012.
10. Feitelson MA and Lee J: Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Lett 252: 157-170, 2007.
11. Taha SE, El-Hady SA, Ahmed TM and Ahmed IZ: Detection of occult HBV infection by nested PCR assay among chronic hepatitis C patients with and without hepatocellular carcinoma. Egypt J Med Hum Genet 14: 353-360, 2013.
12. Li X, Zhang J, Yang Z, Kang J, Jiang S, Zhang T, Chen T, Li M, Lv Q, Chen X, *et al*: The function of targeted host genes determines the oncogenicity of HBV integration in hepatocellular carcinoma. J Hepatol 60: 975-984, 2014.
13. Guerrieri F, Belloni L, Pediconi N and Levrero M: Molecular mechanisms of HBV-associated hepatocarcinogenesis. Semin Liver Dis 33: 147-156, 2013.
14. Rey-Cuille MA, Njouom R, Bekondi C, Seck A, Gody C, Bata P, Garin B, Maylin S, Chartier L, Simon F and Vray M: Hepatitis B virus exposure during childhood in Cameroon, Central African Republic and Senegal after the integration of HBV vaccine in the expanded program on immunization. Pediatr Infect Dis J 32: 1110-1115, 2013.
15. Bonilla Guerrero R and Roberts LR: The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. J Hepatol 42: 760-777, 2005.
16. Bok J, Kim KJ, Park MH, Cho SH, Lee HJ, Lee EJ, Park C and Lee JY: Identification and extensive analysis of inverted-duplicated HBV integration in a human hepatocellular carcinoma cell line. BMB Rep 45: 365-370, 2012.
17. Arzumanyan A, Reis HM and Feitelson MA: Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nat Rev Cancer 13: 123-135, 2013.
18. Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, *et al*: Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet 44: 760-764, 2012.
19. Jiang Z, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, Guan Y, Lee W, Carnevali P, Stinson J, Johnson S, *et al*: The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. Genome Res 22: 593-601, 2012.
20. Amaddeo G, Cao Q, Ladeiro Y, Imbeaud S, Nault JC, Jaoui D, Gaston Mathe Y, Laurent C, Laurent A, Bioulac-Sage P, *et al*: Integration of tumour and viral genomic characterisations in HBV-related hepatocellular carcinomas. Gut 64: 820-829, 2015.
21. Murakami Y, Saigo K, Takashima H, Minami M, Okanoue T, Bréchot C and Paterlini-Bréchot P: Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas. Gut 54: 1162-1168, 2005.
22. Saigo K, Yoshida K, Ikeda R, Sakamoto Y, Murakami Y, Urashima T, Asano T, Kenmochi T and Inoue I: Integration of hepatitis B virus DNA into the myeloid/lymphoid or mixed-lineage leukemia (MLL4) gene and rearrangements of MLL4 in human hepatocellular carcinoma. Hum Mutat 29: 703-708, 2008.
23. Li H and Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754-1760, 2009.
24. Bauer DC: Variant calling comparison CASAVA1.8 and GATK. Nat Prec, 2011.
25. Lim L, Tran BM, Vincan E, Locarnini S and Warner N: HBV-related hepatocellular carcinoma: The role of integration, viral proteins and miRNA. Future Virol 7: 1237-1249, 2012.
26. Tamori A, Nishiguchi S, Kubo S, Narimatsu T, Habu D, Takeda T, Hirohashi K and Shiomi S: HBV DNA integration and HBV-transcript expression in non-B, non-C hepatocellular carcinoma in Japan. J Med Virol 71: 492-498, 2003.
27. Zhao LH, Liu X, Yan HX, Li WY, Zeng X, Yang Y, Zhao J, Liu SP, Zhuang XH, Lin C, *et al*: Genomic and oncogenic preference of HBV integration in hepatocellular carcinoma. Nat Commun 7: 12992, 2016.
28. Zhang Z: Abstract LB-400: The effects of hepatitis B virus integration into the genomes of hepatocellular carcinoma patients. Cancer Res 72 (Suppl 8): LB-400, 2012.
29. Bréchot C, Gozuacik D, Murakami Y and Paterlini-Bréchot P: Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol 10: 211-231, 2000.