VIROLOGICA SINICA 2015, 30 (2): 85-91
DOI 10.1007/s12250-015-3582-7

REVIEW

Genetic and epigenetic alterations in hepatitis B virus-associated hepatocellular carcinoma

Yongjun Tian, Jing-hsiung James Ou✉

Department of Molecular Microbiology and Immunology, University of Southern California Keck School of Medicine, Los Angeles 90033, USA

Hepatitis B virus (HBV) is a major cause of hepatocellular carcinoma (HCC). Its chronic infection can lead to chronic liver inflammation and the accumulation of genetic alterations to result in the oncogenic transformation of hepatocytes. HBV can also sensitize hepatocytes to oncogenic transformation by causing genetic and epigenetic changes of the host chromosomes. HBV DNA can insert into host chromosomes and recent large-scale whole-genome sequencing studies revealed recurrent HBV DNA integrations sites that may play important roles in the initiation of hepatocellular carcinogenesis. HBV can also cause epigenetic changes by altering the methylation status of cellular DNA, the post-translational modification of histones, and the expression of microRNAs. These changes can also lead to the eventual hepatocellular transformation. These recent findings on the genetic and epigenetic alterations of the host chromosomes induced by HBV opened a new avenue for the development of novel diagnosis and treatments for HBV-induced HCC.

KEYWORDS hepatitis B virus (HBV); hepatocellular carcinoma (HCC); genetic alterations; epigenetic regulations; HCC-associated microRNAs

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the second leading cause of cancer deaths (Laursen, 2014). The four major risk factors for HCC are chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus (HCV) infection, alcohol consumption and the exposure to environmental toxins. Among these risk factors, HBV is the most important factor and responsible for over 50% of HCC cases worldwide (El-Serag and Rudolph, 2007).

HBV may induce HCC indirectly via the induction of chronic liver inflammation, which can cause oxidative stress and repeated liver injury and regeneration. This can lead to the accumulation of genetic alternations in hepatocytes and the eventual development of HCC (Cougot et al., 2005). HBV may also alter the redox status in hepatocytes and sensitize them to oxidative stress and environmental factors, leading to chromosomal instability and cellular transformation (Zheng et al., 2007; Na et al., 2011; Wang et al., 2012). Accumulating evidence indicated that HBV could also directly induce genetic and epigenetic alterations of host chromosomes to result in the development of HCC. These genetic and epigenetic alterations may be caused by the integration of HBV DNA, the DNA hypomethylation, the CpG island hypermethylation, and the aberrant expression of microRNAs. In this review, we will discuss recent studies on the genetic and epigenetic alterations in HCCs isolated from HBV patients and the possible roles of these alterations in HBV-induced hepatocarcinogenesis.

Received: 13 March 2015, Accepted: 25 March 2015
Published online: 7 April 2015
✉ Correspondence:
Phone: +1-323-442-1720, Fax: +1-323-442-1721,
Email: jamesou@hsc.usc.edu
ORCID: 0000-0002-8274-5705

GENETIC ALTERATIONS IN HBV-ASSOCIATED HCC

HBV DNA integration in host chromosomes

HBV is an enveloped virus with a circular and partially double-stranded DNA genome of about 3.2 kb. After infecting hepatocytes, the HBV DNA is transported into the nucleus where it is converted to the fully double-stranded and covalently closed circular DNA (cccDNA). The HBV cccDNA is then assembled into a minichromosome by histones, and serves as the template to direct the transcription of viral mRNAs (Levrero et al., 2009). The HBV cccDNA is very stable and may persist for many years in the nucleus of infected hepatocytes. It can also be replenished and amplified by the HBV genomic DNA that has been replicated in the cytoplasm. The persistence of cccDNA in the nucleus as well as the continuous entry of the partially double-stranded viral DNA genome into the nucleus of infected hepatocytes significantly increases the probability of HBV DNA integration into the host chromosomes. The integration of HBV DNA into the host chromosomes was first reported 35 years ago when the HCC tissues isolated from HBV patients were analyzed (Brechot et al., 1980; Chakraborty et al., 1980; Edman et al., 1980). This DNA integration is not an essential step of the viral life cycle, but nevertheless it was frequently detected in HCCs isolated from HBV patients (Sung et al., 2012). It was initially thought that the HBV DNA integrated randomly into the host chromosomes of HCC (Matsubara and Tokino, 1990), but recent studies using the next-generation sequencing, which enables large-scale analysis of HBV DNA integration sites in HCC, led to the finding that there were recurrent HBV DNA insertion sites in the host chromosomes, and many HBV DNA actually integrated within or near repetitive sequences. The genetic loci of recurrent integration sites included *FN1, MLL4, CCNE1, SENP5, ROCK1, ADH1B, CPS1, ESRRG, LRFN2, MYOM1, RAI1, FAR2, ITPR1, IRAK2, NAPK1, MLL2, CCNE1, TP53, CTNNB1, ARID1A, ARID2, AXIN1, LINEs, SINEs, SMAD5, PHACTR4* and *RBFOX1* (Ding et al., 2012; Jiang et al., 2012; Sung et al., 2012; Li et al., 2013). Fujimoto *et al*. reported that 4 of 11 HBV-positive tumor tissues had HBV DNA integrating within or upstream of the human *TERT* (*hTERT*) gene (Fujimoto et al., 2012). Sung *et al*. reported that HBV DNA integration occurred 18 times at *hTERT*, 9 times at *MLL4* and 4 times at *CCNE1* in 81 HCC samples (Sung et al., 2012). The detection of recurrent HBV DNA integration sites in HCC chromosomes indicated a possible role of these integration events infacilitating hepatocarcinogenesis, which led to their positive selection in HCC.

HBV DNA integration may lead to the dysregulated expression of wild-type or truncated viral proteins that affect gene expressions or signaling pathways in the host cell (Kekule et al., 1990; Hai et al., 2014). HBV DNA integration sites in the viral genome are often located with a 1,800-bp region where the HBV enhancer, the X gene and the core protein gene reside (Sung et al., 2012). The integration of HBV DNA may be facilitated by the relaxed circular form of the viral genome, which has free 5' and 3' ends (Shih et al., 1987). The integrated HBV DNA may also exert *cis* and *trans* effects on the host genes. The *cis* effects include the disruption or the induction of the host gene at or near the integration site, and the *trans* effects often result from the production of a truncated viral gene product or the expression of a hybrid transcript containing virus and host DNA sequences that affect the expression of host genes at different locations (Ou and Rutter, 1985; Hai et al., 2014; Lau et al., 2014). HBV DNA integration has been shown to drive the expression of a long interspersed nuclear element (LINE1) repetitive sequence, generating a chimeric HBV X (HBx)-LINE1b transcript (Lau et al., 2014). This chimeric transcript functions as an oncogenic RNA that is capable of stimulating the Wnt/β-catenin signaling pathway. The knockdown of HBx-LINE1b RNA reduced the migratory and invasive abilities of hepatoma cells, whereas the transgene-driven expression of HBx-LINE1b promoted cell migration and invasion. HBx-LINE1b transgenic mice were more likely to develop HCC than the control mice when treated with the carcinogen diethylnitrosamine (Lau et al., 2014). These studies demonstrated that HBV DNA integration could play a positive role in hepatocarcinogenesis.

Genetic mutations

Systematic and comprehensive analyses of large numbers of matched tumor and non-tumor samples with the next-generation whole-genome sequencing had been used to study the molecular mechanism of hepatocarcinogenesis. These studies led to the identification of recurrent somatic mutations of genes in tumor samples, including genes that had not been previously reported. TP53 mutations had been identified in many HCC tissues isolated from HBV patients (for an example, see (Guichard et al., 2012)). Three genes, *CTNNB1*, *AXIN1* and *CDKN2A*, had also been shown to be frequently mutated in HBV-associated HCC (Tao et al., 2011; Fujimoto et al., 2012; Kan et al., 2013). *CTNNB1*, which encodes β-catenin, is the most frequently mutated oncogene in HCC. *CTNNB1* mutations occurred in 32.8% of HCC in one study (Fujimoto et al., 2012), and 62.5% in another study (Kan et al., 2013). The *CTNNB1* mutation rate is about 12% in HBV-related HCC and lower than in HCCs caused by other risk factors (Guichard et al., 2012). The mutations in *CTNNB1* can activate the Wnt/β-catenin pathway (Guichard et al., 2012), and have been suggested to act as a major oncogenic driver for HCC (Kan et al., 2013). *AXIN1* encodes Axin1, which interacts with a number of cellular proteins including β-catenin. It often

displays point mutations and small deletions in HCCs. *CDKN2A*, which encodes $p16^{INK4a}$, a tumor suppressor and negative regulator of the cell cycle, may undergo homozygous deletion and epigenetic silencing in HCCs (Ozturk et al., 2009).

Fujimoto *et al*. sequenced 27 HCCs including 11 samples from HBV patients and 14 samples from HCV patients and identified novel mutations in multiple chromatin regulators genes, including *ARID1A*, *ARID1B*, *ARID2*, *MLL* and *MLL3*, in about 50% of tumors (Fujimoto et al., 2012). There was no significant difference of the genetic mutation profiles between the HCC samples isolated from HBV and HCV patients. *ARID1A*, which encodes a key component of the SWI/SNF chromatin remodeling complex, was also found to be mutated in 13% of HCC specimens isolated from HBV patients in a separate study (Huang et al., 2012). If the expression of these chromatin regulators in HCC cell lines that expressed the wild-type genes were suppressed by siRNA, cell proliferation, invasion and migration were enhanced, supporting the role of these loss-of-function mutations in the development of HCC (Fujimoto et al., 2012; Huang et al., 2012).

The somatic mutations of genes identified in HBV-associated HCCs may be generated due to the chronic liver inflammation in response to HBV infection, the induction of oxidative stress by HBV, the inhibition of DNA-damage repair by HBV and/or the exposure of HBV patients to environmental factors such as carcinogens (Becker et al., 1998; Zheng et al., 2007; Wang et al., 2012). These mutations in HCV-associated HCCs may be generated through similar mechanisms. Once these mutations are generated, they will facilitate the development of HCC.

EPIGENETIC ALTERATIONS IN HBV-SSOCIATED HCC

Epigenetic modifications are important for regulating gene expression, and their abnormalities can lead to cellular transformation. Epigenetic modifications include DNA methylation and a wide spectrum of post-translational histone modifications. The importance of epigenetic alterations in the development of HCC is being increasingly recognized.

DNA methylation

Aberrant DNA methylation patterns, which can cause the alteration of gene expression profiles, have been found in many human cancers including HCC. Global DNA hypomethylation such as in repetitive sequences and transposable elements can cause chromosomal instability and mutations, and the hypermethylation typically at the clusters of CpG dinucleotides, known as CpG islands (CGIs), in the promoters of genes can result in the silencing of tumor suppressor genes (Lee et al., 2014). HBV infection could affect the methylation of $p16^{INK4A}$, $p21^{WAF1/CIP1}$, *RASSF1A* (Ras association domain family member 1), *GSTP1* and *CDH1* genes (Liang et al., 2014). $p16^{INK4A}$, $p21^{WAF1/CIP1}$ and *RASSF1A* are involved in the cell cycle control and the suppression of their expression can lead to cellular proliferation. *GSTP1* encodes glutathione-s-transferase P1, which inhibits oxidative damage to the cell and inactivates electrophilic carcinogens. *CDH1* encodes E-cadherin and is involved in cell adhesion. Its loss of expression has been implicated in cancer progression and metastasis. Another report found *ASPP1* and *ASPP2* genes, which play important roles in apoptosis, are frequently down-regulated by DNA methylation in HBV-associated HCC (Zhao et al., 2010). By using mice grafted with human hepatocytes, Okamoto *et al*. also found that DNA methylation increased in human hepatocytes in a time-dependent manner during HBV infection (Okamoto et al., 2014).

Cellular DNA methylation may be altered by HBV infection via DNA methyltransferases (DNMTs). HBx has been shown to up-regulate *DNMT1*, *DNMT3A1* and *DNMT3A2* in cell cultures (Park et al., 2007). These DNMTs mediate the regional hypermethylation of tumor suppressor genes. HBx also suppresses the expression of *DNMT3B* to cause the global hypomethylation of satellite 2 repeat sequences (Park et al., 2007). Severe hypomethylation of intragenic CGIs was also observed in the HBx transgenic mouse liver before the development of HCC in these mice. These CGIs are normally highly methylated (mCGIs) by the DNMT3L complex and associated with active gene expressions. HBx together with histone deacetylase 1 (HDAC1) could bind to the promoter of *DNMT3L* to inhibit its expression, leading to the hypomethylation of mCGIs and the down-regulation of many developmental regulators that control tumorigenesis (Lee et al., 2014). Curiously, although HBx was found to up-regulate *DNMT3A* in cell cultures (Park et al., 2007), it inhibited the expression of *DNMT3A* in the mouse liver (Lee et al., 2014). The reason for this discrepancy is unclear.

Post-translational modifications of histones

The acetylation of histones plays an important role in cancer development and progression. The expression of histone deacetylases (HDACs) is altered in many human cancers, including HCC (Weichert, 2009). In a study of 12 paired HCC and its surrounding non-tumor liver tissues isolated from HBV patients, the expression of HDAC1 was found to be increased in 10 of the HCC tissues (Yoo et al., 2008). As the expression of *HDAC1* was also higher in the liver of HBx-transgenic mice, the HBx protein was apparently sufficient for the induction of

HDAC1 expression. HDAC1 can also suppress $p21^{WAF1/CIP1}$ expression by interacting with a Sp1-binding site in the $p21^{WAF1/CIP1}$ promoter (Xie et al., 2012). The inactivation of HDAC1 induced the regression of tumor growth and caused the caspase-independent autophagic cell death. The HDAC1 inactivation selectively induced both $p21^{WAF1/CIP1}$ and $p27^{Kip1}$ expressions, and simultaneously suppressed the expression of cyclin D1 and CDK2 (Xie et al., 2012). The inactivation of HDAC1 also resulted in the hypophosphorylation of the tumor suppressor pRb, which then inactivated the E2F/DP1 transcription factor and inhibited the G1/S transition of the cell cycle. Besides HDAC1, HDAC2 and HDCA3 are also over-expressed in HCC (Wu et al., 2010).

MICRORNAS IN HBV-ASSOCIATED HCC

MicroRNAs (miRNAs) are small non-coding RNAs with a size of 19–25 nucleotides. They can regulate gene expression usually by gene silencing via translational repression or the degradation of mRNAs. There are more than 1000 miRNAs that have been identified in human cells. Some of them can be regulated by HBV and have been implicated in HBV-induced hepatocarcinogenesis. For examples, *miR-21* and the *miR-17-92* polycistron, which expresses miR-17, 18a, 19a/b, 20a and 92a, were found to be highly expressed in all of the HCCs isolated from HBV patients, and the suppression of their expression in HepG2 hepatoblastoma cells led to the reduction of the tumorigenicity of this cell line (Connolly et al., 2008). In another study, *miR-545* and *miR-374a*, which were encoded in the intron of the Ftx long non-coding RNA, were also found to be up-regulated in HBV-associated HCCs. These two miRNAs could be induced by either HBV or HBx in cell cultures, and could promote cell proliferation, migration and invasion (Zhao et al., 2014). Similarly, *miR-224* was found to be up-regulated in HBV-associated HCC (Gao et al., 2011; Lan et al., 2014). This microRNA could target *Smad 4* to promote tumorigenesis (Lan et al., 2014). In contrast, *miR-145* and *miR-199b* were down-regulated in pre-malignant tumor nodules and HCCs of HBV patients and the restoration of miR-145 expression was found to reduce the proliferation, migration and invasiveness of HepG2 and Hep3B cells (Gao et al., 2011). These studies indicated that HBV could induce hepatocarcinogenesis via the regulation of expression of microRNAs.

In addition to *miR-545* and *miR-374a* mentioned above, HBx, the regulatory protein of HBV, can also directly and indirectly regulate the expression of other microRNAs. HBx has been shown to inactivate p53, which reduces the expression level of *miRNA-23a*, *miRNA-34*, *miRNA-125b*, *miRNA-132*, *miRNA-148a*, *miRNA-192* and *miRNA-200*, and promote a more aggressive cancer phenotype (Zhang et al., 2009; Tao et al., 2011; Scisciani et al., 2012; Yang et al., 2012; Han et al., 2013; Noh et al., 2013). HBx can also down-regulate Let-7a to increase the Stat3 expression and promote cell prolifera-

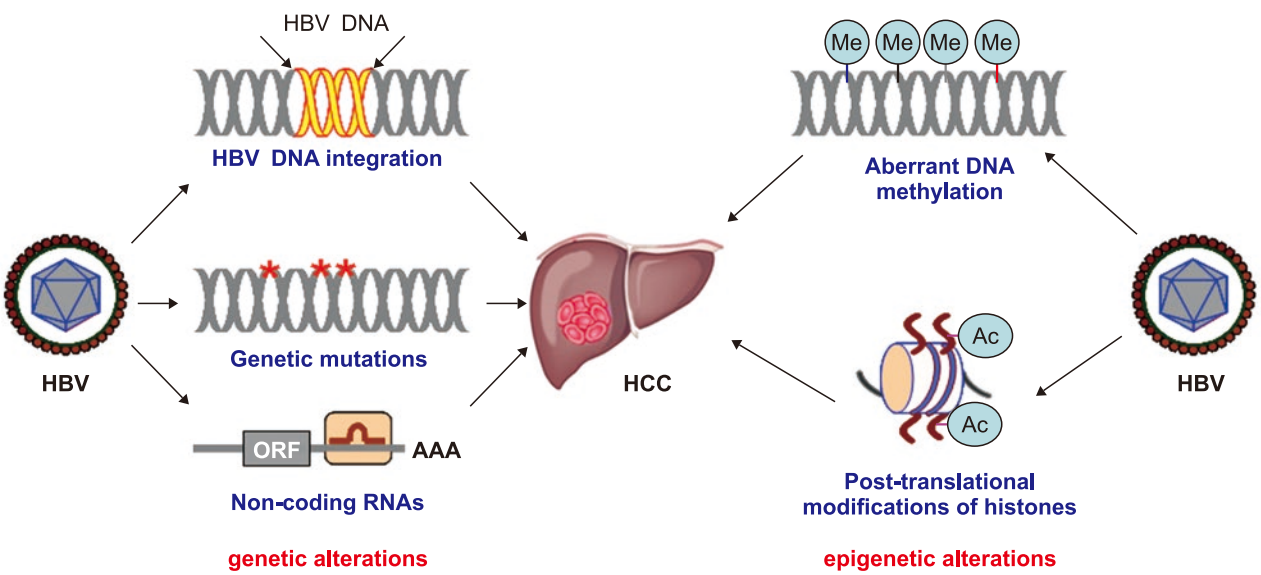

Figure 1. Genetic and epigenetic alterations induced by HBV. HBV can cause genetic alterations of its host cells by inserting its genomic DNA into host chromosomes, inducing DNA mutations and affecting the expression of non-coding RNAs. HBV can also cause epigenetic alterations of its host cells by altering methylation patterns of host DNA and post-translational modifications of histones. These genetic and epigenetic alterations can lead to the initiation and promote the progression of hepatocellular carcinogenesis.

tion (Wang et al., 2010). It can also down-regulate *miRNA-152*, which is frequently down-regulated in HBV-associated HCC. The expression level of *miRNA-152* is inversely correlated with the expression level of DNMT1 (Huang et al., 2010), which, as mentioned above, methylates the promoters of many tumor suppressor genes. The over-expression of *miR-152* resulted in the significant reduction of DNMT1 mRNA and protein levels, and its silencing induced the global DNA hypermethylation and increased the methylation levels of two tumor suppressor genes, *GSTP1* and *E-cadherin* (Huang et al., 2010). HBx could also down-regulate *miR-101*, which targets *DNMT3A*, to increase DNA methylation of several tumor suppressor genes and to suppress their expression (Wei et al., 2013). The *miR-101* also negatively regulates the expression of *EZH2* (enhancer of zeste homolog 2), a histone lysine N-methyltransferase, in HCC (Xu et al., 2014). *EZH2* methylates lysine-9 and lysine-27 of histone 3 (i.e., H3K9me and H3K27me) to repress the expression of target genes. The down-regulation of *miR-101* by HBx can therefore increase the expression level of *EZH2* and suppress the expression of its target genes. HBx could also up-regulate *miRNA-21*, which is known to be an oncogenic miRNA that targets *PTEN*, *PDCD4* and *RECK*-tumor suppressor genes, to promote hepatocarcinogenesis (Liu et al., 2010).

CONCLUSION

HBV-associated HCC frequently contains genetic and epigenetic alterations. In this review, we summarized recent findings of these alterations. HBV DNA can integrate into host chromosomes to alter the expression of cellular genes and sensitize hepatocytes to oncogenic transformation. HBV can also induce DNA hypomethylation and hypermethylation to regulate the expression of cellular oncogenes and tumor suppressor genes. It may also affect the post-translational modification of histones and the expression of miRNAs to alter gene expression profiles in hepatocytes. These genetic and epigenetic alterations caused by HBV infection, which are illustrated in Figure 1, are believed to play important roles in the development of HCC in HBV patients and may serve as the targets for the development of novel therapeutic interventions for treating HBV-associated HCC.

ACKNOWLEDGMENTS

This work was supported by the NIH grants DK100257 and DK177337.

COMPLIANCE WITH ETHICS GUIDELINES

The authors declared that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

REFERENCES

Becker SA, Lee TH, Butel JS, Slagle BL. 1998. Hepatitis b virus x protein interferes with cellular DNA repair. J Virol, 72: 266–272.

Brechot C, Pourcel C, Louise A, Rain B, Tiollais P. 1980. Presence of integrated hepatitis b virus DNA sequences in cellular DNA of human hepatocellular carcinoma. Nature, 286: 533–535.

Chakraborty PR, Ruiz-Opazo N, Shouval D, Shafritz DA. 1980. Identification of integrated hepatitis b virus DNA and expression of viral rna in an hbsag-producing human hepatocellular carcinoma cell line. Nature, 286: 531–533.

Connolly E, Melegari M, Landgraf P, Tchaikovskaya T, Tennant BC, Slagle BL, Rogler LE, Zavolan M, Tuschl T, Rogler CE. 2008. Elevated expression of the mir-17-92 polycistron and mir-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype. Am J Pathol, 173: 856–864.

Cougot D, Neuveut C, Buendia MA. 2005. Hbv induced carcinogenesis. J Clin Virol, 34 Suppl 1: S75–78.

Ding D, Lou X, Hua D, Yu W, Li L, Wang J, Gao F, Zhao N, Ren G, Li L, Lin B. 2012. Recurrent targeted genes of hepatitis b virus in the liver cancer genomes identified by a next-generation sequencing-based approach. PLoS Genet, 8: e1003065.

Edman JC, Gray P, Valenzuela P, Rall LB, Rutter WJ. 1980. Integration of hepatitis b virus sequences and their expression in a human hepatoma cell. Nature, 286: 535–538.

El-Serag HB, Rudolph KL. 2007. Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. Gastroenterology, 132: 2557–2576.

Fujimoto A, Totoki Y, Abe T, Boroevich KA, Hosoda F, Nguyen HH, Aoki M, Hosono N, Kubo M, Miya F, Arai Y, Takahashi H, Shirakihara T, Nagasaki M, Shibuya T, Nakano K, Watanabe-Makino K, Tanaka H, Nakamura H, Kusuda J, Ojima H, Shimada K, Okusaka T, Ueno M, Shigekawa Y, Kawakami Y, Arihiro K, Ohdan H, Gotoh K, Ishikawa O, Ariizumi S, Yamamoto M, Yamada T, Chayama K, Kosuge T, Yamaue H, Kamatani N, Miyano S, Nakagama H, Nakamura Y, Tsunoda T, Shibata T, Nakagawa H. 2012. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet, 44: 760–764.

Gao P, Wong CC, Tung EK, Lee JM, Wong CM, Ng IO. 2011. Deregulation of microrna expression occurs early and accumulates in early stages of hbv-associated multistep hepatocarcinogenesis. J Hepatol, 54: 1177–1184.

Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, Clement B, Balabaud C, Chevet E, Laurent A, Couchy G, Letouze E, Calvo F, Zucman-Rossi J. 2012. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet, 44: 694–698.

Hai H, Tamori A, Kawada N. 2014. Role of hepatitis b virus DNA integration in human hepatocarcinogenesis. World J Gastroenterol, 20: 6236–6243.

Han H, Sun D, Li W, Shen H, Zhu Y, Li C, Chen Y, Lu L, Li W, Zhang J, Tian Y, Li Y. 2013. A c-myc-microrna functional feedback loop affects hepatocarcinogenesis. Hepatology, 57: 2378–2389.

Huang J, Deng Q, Wang Q, Li KY, Dai JH, Li N, Zhu ZD, Zhou B, Liu XY, Liu RF, Fei QL, Chen H, Cai B, Zhou B, Xiao HS, Qin

LX, Han ZG. 2012. Exome sequencing of hepatitis b virus-associated hepatocellular carcinoma. Nat Genet, 44: 1117–1121.
Huang J, Wang Y, Guo Y, Sun S. 2010. Down-regulated micro-rna-152 induces aberrant DNA methylation in hepatitis b virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. Hepatology, 52: 60–70.
Jiang Z, Jhunjhunwala S, Liu J, Haverty PM, Kennemer MI, Guan Y, Lee W, Carnevali P, Stinson J, Johnson S, Diao J, Yeung S, Jubb A, Ye W, Wu TD, Kapadia SB, de Sauvage FJ, Gentleman RC, Stern HM, Seshagiri S, Pant KP, Modrusan Z, Ballinger DG, Zhang Z. 2012. The effects of hepatitis b virus integration into the genomes of hepatocellular carcinoma patients. Genome Res, 22: 593–601.
Kan Z, Zheng H, Liu X, Li S, Barber TD, Gong Z, Gao H, Hao K, Willard MD, Xu J, Hauptschein R, Rejto PA, Fernandez J, Wang G, Zhang Q, Wang B, Chen R, Wang J, Lee NP, Zhou W, Lin Z, Peng Z, Yi K, Chen S, Li L, Fan X, Yang J, Ye R, Ju J, Wang K, Estrella H, Deng S, Wei P, Qiu M, Wulur IH, Liu J, Ehsani ME, Zhang C, Loboda A, Sung WK, Aggarwal A, Poon RT, Fan ST, Wang J, Hardwick J, Reinhard C, Dai H, Li Y, Luk JM, Mao M. 2013. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Genome Res, 23: 1422–1433.
Kekule AS, Lauer U, Meyer M, Caselmann WH, Hofschneider PH, Koshy R. 1990. The pres2/s region of integrated hepatitis b virus DNA encodes a transcriptional transactivator. Nature, 343: 457–461.
Lan SH, Wu SY, Zuchini R, Lin XZ, Su IJ, Tsai TF, Lin YJ, Wu CT, Liu HS. 2014. Autophagy suppresses tumorigenesis of hepatitis b virus-associated hepatocellular carcinoma through degradation of microrna-224. Hepatology, 59: 505–517.
Lau CC, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, Lai PB, Chan HL, To KF, Chan TF, Wong N. 2014. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. Cancer Cell, 25: 335–349.
Laursen L. 2014. A preventable cancer. Nature, 516: S2–3.
Lee SM, Lee YG, Bae JB, Choi JK, Tayama C, Hata K, Yun Y, Seong JK, Kim YJ. 2014. Hbx induces hypomethylation of distal intragenic cpg islands required for active expression of developmental regulators. Proc Natl Acad Sci U S A, 111: 9555–9560.
Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. 2009. Control of cccdna function in hepatitis b virus infection. J Hepatol, 51: 581–592.
Li W, Zeng X, Lee NP, Liu X, Chen S, Guo B, Yi S, Zhuang X, Chen F, Wang G, Poon RT, Fan ST, Mao M, Li Y, Li S, Wang J, Jianwang, Xu X, Jiang H, Zhang X. 2013. Hivid: An efficient method to detect hbv integration using low coverage sequencing. Genomics, 102: 338–344.
Liang RP, Na H, Li ZF, Ji FP, Jiang SW. 2014. Epigenetic mechanism involved in the hbv/hcv-related hepatocellular carcinoma tumorigenesis. Curr Pharm Des, 20: 1715–1725.
Liu C, Yu J, Yu S, Lavker RM, Cai L, Liu W, Yang K, He X, Chen S. 2010. Microrna-21 acts as an oncomir through multiple targets in human hepatocellular carcinoma. J Hepatol, 53: 98–107.
Matsubara K, Tokino T. 1990. Integration of hepatitis b virus DNA and its implications for hepatocarcinogenesis. Mol Biol Med, 7: 243–260.
Na B, Huang Z, Wang Q, Qi Z, Tian Y, Lu CC, Yu J, Hanes MA, Kakar S, Huang EJ, Ou JH, Liu L, Yen TS. 2011. Transgenic expression of entire hepatitis b virus in mice induces hepatocarcinogenesis independent of chronic liver injury. PLoS One, 6: e26240.
Noh JH, Chang YG, Kim MG, Jung KH, Kim JK, Bae HJ, Eun JW, Shen Q, Kim SJ, Kwon SH, Park WS, Lee JY, Nam SW. 2013. Mir-145 functions as a tumor suppressor by directly targeting histone deacetylase 2 in liver cancer. Cancer Lett, 335: 455–462.
Okamoto Y, Shinjo K, Shimizu Y, Sano T, Yamao K, Gao W, Fujii M, Osada H, Sekido Y, Murakami S, Tanaka Y, Joh T, Sato S, Takahashi S, Wakita T, Zhu J, Issa JP, Kondo Y. 2014. Hepatitis virus infection affects DNA methylation in mice with humanized livers. Gastroenterology, 146: 562–572.
Ou J, Rutter WJ. 1985. Hybrid hepatitis b virus-host transcripts in a human hepatoma cell. Proc Natl Acad Sci U S A, 82: 83–87.
Ozturk M, Arslan-Ergul A, Bagislar S, Senturk S, Yuzugullu H. 2009. Senescence and immortality in hepatocellular carcinoma. Cancer Lett, 286: 103–113.
Park IY, Sohn BH, Yu E, Suh DJ, Chung YH, Lee JH, Surzycki SJ, Lee YI. 2007. Aberrant epigenetic modifications in hepatocarcinogenesis induced by hepatitis b virus x protein. Gastroenterology, 132: 1476–1494.
Scisciani C, Vossio S, Guerrieri F, Schinzari V, De Iaco R, D'Onorio de Meo P, Cervello M, Montalto G, Pollicino T, Raimondo G, Levrero M, Pediconi N. 2012. Transcriptional regulation of mir-224 upregulated in human hccs by nfkappab inflammatory pathways. J Hepatol, 56: 855–861.
Shih C, Burke K, Chou MJ, Zeldis JB, Yang CS, Lee CS, Isselbacher KJ, Wands JR, Goodman HM. 1987. Tight clustering of human hepatitis b virus integration sites in hepatomas near a triple-stranded region. J Virol, 61: 3491–3498.
Sung WK, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, Mulawadi FH, Wong KF, Liu AM, Poon RT, Fan ST, Chan KL, Gong Z, Hu Y, Lin Z, Wang G, Zhang Q, Barber TD, Chou WC, Aggarwal A, Hao K, Zhou W, Zhang C, Hardwick J, Buser C, Xu J, Kan Z, Dai H, Mao M, Reinhard C, Wang J, Luk JM. 2012. Genome-wide survey of recurrent hbv integration in hepatocellular carcinoma. Nat Genet, 44: 765–769.
Tao Y, Ruan J, Yeh SH, Lu X, Wang Y, Zhai W, Cai J, Ling S, Gong Q, Chong Z, Qu Z, Li Q, Liu J, Yang J, Zheng C, Zeng C, Wang HY, Zhang J, Wang SH, Hao L, Dong L, Li W, Sun M, Zou W, Yu C, Li C, Liu G, Jiang L, Xu J, Huang H, Li C, Mi S, Zhang B, Chen B, Zhao W, Hu S, Zhuang SM, Shen Y, Shi S, Brown C, White KP, Chen DS, Chen PJ, Wu CI. 2011. Rapid growth of a hepatocellular carcinoma and the driving mutations revealed by cell-population genetic analysis of whole-genome data. Proc Natl Acad Sci U S A, 108: 12042–12047.
Wang Q, Na B, Ou JH, Pulliam L, Yen TS. 2012. Hepatitis b virus alters the antioxidant system in transgenic mice and sensitizes hepatocytes to fas signaling. PLoS One, 7: e36818.
Wang Y, Lu Y, Toh ST, Sung WK, Tan P, Chow P, Chung AY, Jooi LL, Lee CG. 2010. Lethal-7 is down-regulated by the hepatitis b virus x protein and targets signal transducer and activator of transcription 3. J Hepatol, 53: 57–66.
Wei X, Xiang T, Ren G, Tan C, Liu R, Xu X, Wu Z. 2013. Mir-101 is down-regulated by the hepatitis b virus x protein and induces aberrant DNA methylation by targeting DNA methyltransferase 3a. Cell Signal, 25: 439–446.
Weichert W. 2009. Hdac expression and clinical prognosis in human malignancies. Cancer Lett, 280: 168–176.
Wu LM, Yang Z, Zhou L, Zhang F, Xie HY, Feng XW, Wu J, Zheng SS. 2010. Identification of histone deacetylase 3 as a biomarker for tumor recurrence following liver transplantation in hbv-associated hepatocellular carcinoma. PLoS One, 5: e14460.
Xie HJ, Noh JH, Kim JK, Jung KH, Eun JW, Bae HJ, Kim MG, Chang YG, Lee JY, Park H, Nam SW. 2012. Hdac1 inactivation induces mitotic defect and caspase-independent autophagic cell death in liver cancer. PLoS One, 7: e34265.
Xu L, Beckebaum S, Iacob S, Wu G, Kaiser GM, Radtke A, Liu

C, Kabar I, Schmidt HH, Zhang X, Lu M, Cicinnati VR. 2014. Microrna-101 inhibits human hepatocellular carcinoma progression through ezh2 downregulation and increased cytostatic drug sensitivity. J Hepatol, 60: 590–598.

Yang P, Li QJ, Feng Y, Zhang Y, Markowitz GJ, Ning S, Deng Y, Zhao J, Jiang S, Yuan Y, Wang HY, Cheng SQ, Xie D, Wang XF. 2012. Tgf-beta-mir-34a-ccl22 signaling-induced treg cell recruitment promotes venous metastases of hbv-positive hepatocellular carcinoma. Cancer Cell, 22: 291–303.

Yoo YG, Na TY, Seo HW, Seong JK, Park CK, Shin YK, Lee MO. 2008. Hepatitis b virus x protein induces the expression of mta1 and hdac1, which enhances hypoxia signaling in hepatocellular carcinoma cells. Oncogene, 27: 3405–3413.

Zhang X, Liu S, Hu T, Liu S, He Y, Sun S. 2009. Up-regulated microrna-143 transcribed by nuclear factor kappa b enhances hepatocarcinoma metastasis by repressing fibronectin expression. Hepatology, 50: 490–499.

Zhao J, Wu G, Bu F, Lu B, Liang A, Cao L, Tong X, Lu X, Wu M, Guo Y. 2010. Epigenetic silence of ankyrin-repeat-containing, sh3-domain-containing, and proline-rich-region- containing protein 1 (aspp1) and aspp2 genes promotes tumor growth in hepatitis b virus-positive hepatocellular carcinoma. Hepatology, 51: 142–153.

Zhao Q, Li T, Qi J, Liu J, Qin C. 2014. The mir-545/374a cluster encoded in the ftx lncrna is overexpressed in hbv-related hepatocellular carcinoma and promotes tumorigenesis and tumor progression. PLoS One, 9: e109782.

Zheng Y, Chen WL, Louie SG, Yen TS, Ou JH. 2007. Hepatitis b virus promotes hepatocarcinogenesis in transgenic mice. Hepatology, 45: 16–21.