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Review article

Current updates on the molecular and genetic signals as diagnostic and therapeutic targets for hepatitis B virus-associated hepatic malignancy

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ABSTRACT

Liver cancer caused by the hepatitis B virus (HBV) is the third most common cancer-related cause of death worldwide. Early detection of HBV-caused hepatic tumors increases the likelihood of a successful cure. Molecular and genetic signals are becoming more and more recognized as possible indicators of HBV-associated hepatic malignancy and of how well a treatment is working. As a result, we have discussed the current literature on molecular and genetic sensors, including extracellular vesicle microRNAs (EV-miRNAs), long non-coding circulating RNAs (lncRNAs), extracellular vesicles (EVs), and cell free circulating DNA (cfDNA), for the diagnosis and forecasting of HBV-related hepatic cancer. Extracellular vesicle microRNAs such as miR-335-5p, miR-172-5p, miR-1285-5p, miR-497-5p, miR-636, miR-187-5p, miR-223-3p, miR-21, miR-324-3p, miR-210-3p, miR-718, miR-122, miR-522, miR-0308-3p, and miR-375 are essential for the posttranscriptional regulation of oncogenes in hepatic cells as well as the epigenetic modulation of many internal and external signaling pathways in HBV-induced hepatic carcinogenesis. LncRNAs like lnc01977, HULC (highly up-regulated in liver cancer), MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), and HOTAIR (hox transcript antisense intergenic RNA) have been demonstrated to control hepatic-tumors cell growth, relocation, encroachment, and cell death resiliency. They are also becoming more and more involved in immune tracking, hepatic shifting, vasculature oversight, and genomic destabilization. EVs are critical mediators involved in multiple aspects of liver-tumors like angiogenesis, immunology, tumor formation, and the dissemination of malignant hepatocytes. Furthermore, cfDNA contributes to signals associated with tumors, including mutations and abnormal epigenetic changes during HBV-related hepatic tumorigenesis.

1. Introduction

Liver cancer is the second-greatest cause of cancer-related deaths globally, and hepatitis B virus (HBV)-induced hepatocellular carcinoma (HCC) accounts for over 90 % of all primary liver cancer cases [1]. The prevalence of HCC is rising today and may approach

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1 million cases annually [2]. Patients with chronic HBV infection are nearly 100 times more likely to develop HCC, according to observations from prospective cohort studies. HCC is the third leading cause of cancer-related death and the fifth most frequent cancer in the world [3–5].

HBV promotes hepatic malignancy via the incorporation of viral DNA into host genes, which allows the abnormal proliferation, expansion, and infiltration of hepatocytes in the host. Genomic instability may emerge from the integration of HBV DNA into the human genome. Cancer-related genes have been identified to have HBV incorporated into them [6]. The viral HBV genome translated protein products have been linked to accelerating carcinogenesis [7].

Prompt identification of HBV-related HCC can enhance clinical judgment and patient results. Molecular and genetic diagnostic signals or indicators are very beneficial for the development of therapies as well as for early diagnosis [8].

Currently, there are various types of molecular and genetic indicators, including extracellular vesicles (EVs), which are a diverse variety of nanometer-sized substances secreted by tissues during healthy and unhealthy states. The genome, lipids, amino acids, compounds called metabolites, microRNAs (miRNAs), and non-coding RNA make up the EV merchandise, which can change an inflammatory reaction and hence influence the body's defenses. miRNAs connected to EVs play a role in the pathophysiology of HBV-induced hepatic malignancy [9].

On the other hand, miRNAs function as post-transcriptional genetic mufflers, regulating the expression of the targeted mRNAs jointly. This regulatory function allows molecules to be translated precisely. The intrinsically random character of gene expression has been utilized to clarify the supplementary significance of miRNA as moderate inhibitors. Because miRNAs may be useful as signals for cancer treatment and identification, experts are paying more and more attention to them. The expression of miRNAs that may be implicated in the formation of hepatic carcinoma is seriously affected by HBV. Owing to this, miRNAs have been used as pinpoint treatment targets and indicators for early HCC identification [8,10].

In addition, cell-free DNA (cfDNA) is developing as a noninvasive diagnostic tool for a variety of malignancies, including malignancies of the liver brought on by HBV. The cfDNA extracted from hepatic malignant cells is also known as ctDNA. In other words, the DNA that tumors discharge into the bloodstream is known as cell-free tumor DNA (ctDNA), and it is a potential tumor identifier with a wide range of uses. Numerous studies have been conducted in hepatic tumors to examine the relationships between the quantity, genetic mutation rate, epigenetic changes of ctDNA, malignancy's stress actions, and outlook [11,12].

This review will summarize current advances in the diagnostic, therapeutic, and prognostic signals from their molecular and genetic point of view in HBV-related hepatic malignancy.

1.1. Extracellular vesicle microRNAs (EV-miRNAs) as diagnostic and therapeutic targets for HBV-associated hepatic malignancy

Numerous studies have reported markedly various levels of extracellular vesicle (EV) miRNAs (micro-ribonucleic acids) in HBV-associated hepatic malignancy. MicroRNAs, also known as small non-coding RNAs, play a crucial role in the posttranscriptional control of genes in host cells as well as the epigenetic control of several internal and external signaling mechanisms in hepatic tumorigenesis. In addition, intrahepatic miRNAs are encapsulated within EVs and expelled from damaged hepatic cells to additionally facilitate the persistence, expansion, and infiltration of adjacent cells [13].

To regulate viral growth and pathogenicity, miRNAs can alter epigenetic sequences and stimulate immune responses. The replication of viruses and the immune system's antiviral treatment can be regulated by intracellular miRNAs. In order to produce a setting that is conducive to viral evolution, HBV have the ability to generate unique miRNAs and modify their cell's miRNome. Because of these intricate functions, miRNAs are currently examined more frequently as potential signals for the detection, prognosis, and management of hepatic malignancy brought on by HBV [14].

EV miRNA concentrations are examined using real-time quantification via polymerase chain reaction (PCR) microarray, and EV-miRNA quantities are higher among patients with substantial liver damage as opposed to healthy controls as well as those with cirrhosis with decompensated. The inclusion of EV miR-335-5p increased the immunological reliability in forecasting the advancement of catastrophic liver injury caused by chronic HBV infection to cirrhosis that has been decompensated, whereas the combined presence of new-miR-172-5p and miR-1285-5p in blood EVs assisted with anticipating the development of the normal controls to serious hepatic cancer [15].

In cells with hepatic malignancy, as opposed to healthy cells, the concentrations of miR-497-5p expression are reduced, while the target genes such as ACTG1 (actin gamma 1), CSNK1D (casein kinase 1 delta), PPP1CC (protein phosphatase 1 catalytic subunit gamma), and BIRC5 (baculoviral IAP repeat containing 5) are increased. Greater transcription of the four target genes and reduced expression of miR-497-5p are substantially correlated with larger hepatic malignant dimensions. Additionally, an individual's general survival is poorer in those with greater amounts of the targeted genes and less expression of miR-497-5p. The degree of expression of miR-497-5p is strongly linked with the advancement of hepatic malignancy and a poor outcome for patients, suggesting that miR-497-5p may have an inhibitory effect on hepatic cancer. As a result, miR-497-5p might be a particular target of therapy for hepatic malignancy [16].

miRNAs that circulate are thought to be potent diagnostic indicators for HBV-related HCC and represent a new development in fluid investigations. Different machine learning techniques as well as single univariate analysis have been used in the serum miRNA detection process to pinpoint 2/20 (0.01 %) circulating miRNAs (circRNAs) such as miR-636 and miR-187-5p that were discovered simultaneously and that demonstrated an excellent level of efficiency in determining the diagnosis of patients with HBV-HCC [17].

In the previous investigation, the size of cancer and its stage were negatively correlated with serum miR-223-3p. When examined alongside matched adjoining non-tumor cells, the expression of miR-223-3p varied between malignant tissues. It has been demonstrated that miR-223 is essential for natural immunity and that deregulation of its transcription plays a role in the pathophysiology of

hepatic malignancy. According to bioinformatics examination, MiR-223p down-regulates target genes including FOXO3 (Forkhead Box O3), LMO2 (LIM Domain Only 2), and KAT6A (lysine acetyltransferase 6A). Furthermore, for individuals with HBV-associated liver cancer, floating miRNA-223-3p may offer a unique diagnostic and therapeutic indicator [18,19].

The prior study of circRNAs from HCC individuals and HBV silent carriers suggested that circRNA1002 might be a reliable serum predictor for HCC. These results may contribute to the development of an improved test for the early detection of HCC [20]. As potential therapeutic targets and prospective markers, m^6A (N^6-methyladenosine) RNA methylation promoters are also used to identify and diagnose HCC associated with HBV patients in the initial phases of the illness [21].

According to a study, it was discovered that those diagnosed with HCC had greater levels of serum miR-21 than both healthy individuals as well as those with persistent HBV infection. Additionally, miRNA-21 was found to be enhanced in the serum EV portion of patients with HCC compared with the EV-decreased serum component. Progressive cancer stages and cirrhosis have been linked to elevated levels of miRNA-21 expression. Compared to miR-21 in the entire serum, EV-related miR-21 has been shown to have a greater detection specificity and reliability [22,23]. An elevated-serum miR-324-3p could also be an intriguing marker for the identification and outlook of HCC associated with HBV as well as a factor in the development of HBV-caused HCC [24].

Particularly in HBV-related HCC, there were increased levels of miR-210-3p, which controlled several target genes, including HBx, in HBV-positive cell lines with HCC. In HBV-positive HCC cells, the level of transcription of MiR-210-3p is increased while the levels of its target genes are down-regulated. However, suppression of miR-210-3p increased the production of its intended gene. Furthermore, HBx transcription in HBV-infected cells is regulated by miR-210-3p. Hence, miR-210-3p may be a key indicator of hepatocarcinogenesis associated with HBV and blocking miR-210-3p may stop HBV infection from causing hepatic malignancy [25].

Moreover, since miR-3 inhibits the production of new HBV virus particles, the presence of this miRNA likely contributes to the development of a persistent HBV infection. Therefore, adding miR-3 to a miRNA detection index may assist in identifying the role that intrahepatic viral replication plays in the emergence of hepatocyte inflammation and causes hepatic cancer [26]. MiR-3 can control the replication of the host's genes. By preventing PPM1A (protein phosphatase 1A) from translating, miR-3 suppressed PPM1A. PPM1A suppression encourages the expansion of hepatocyte cells linked to the growth of liver cancer [27].

When contrasted with individuals lacking occurrence, those with HCC who experienced a recurrence of cancer following the transplantation of their livers had lower expression of miR-718 in their sera EVs, and lower concentrations of this miRNA were linked to the severity of HCC tumors based on the previous study [28]. PTEN (phosphatase and tensin logue) activity is down-regulated as a result of miR-718 upregulation [29].

Furthermore, miR-122 may be important in the pathophysiology of liver disorders associated with HBV. According to Western blot and reverse transcription quantitative PCR investigations, miR-122 can attach selectively to the 3′ untranslated region (3′UTR) of APOBEC2 (apolipoprotein B mRNA-editing enzyme catalytic subunit 2) and suppress its production. This could lead to the development of carcinoma of the hepatocytes. The results of this investigation supported the hypothesis that employing microRNA-122 for this viral disease's diagnosis and therapy could be a successful strategy [30,31].

On the other hand, in HBV-associated HCC, miR-522 was considerably elevated and linked to a much worse long-term outlook. When miR-522 was silenced, its impacts were lessened, though miR-522 up-regulation increased cell growth and cellular advancement. The target genes of miR-522 included Dickkopf-1 (DKK1) and SFRP2 (secreted frizzled-related protein 2), whose expression in cancer cells had an opposite relationship with that of miR-522. The existence of Wnt (wingless-related integration site) signaling pathway inhibitors, including DKK1 and SFRP2 suggests that miR-522 promotes the evolution of HCC by turning on the signaling pathway Wnt. miR-522 may be a useful target for the treatment of HCC [32].

When juxtaposed with HBV-negative HCC tissues and nearby healthy liver cells, miR-0308-3p significantly decreased in HBV-positive HCC tissues and HCC malignant cell types. By specifically targeting CDK6 (cyclin-dependent kinases 6), miR-0308-3p overexpression significantly inhibited HCC cell growth and caused G1/S (GAP 1/Synthesis) cell cycle stoppage. These outcomes may be therapeutically beneficial in the development of novel treatment targets since they unveil an entirely new molecular explanation for the triggering of the G1/S block in HCC [33].

Based on solexa sequencing and quantitative real-time polymerase chain reaction (qRT-PCR) analysis, miR-375 is also preferentially generated in HBV-induced HCC, and it can be used as a biomarker to distinguish HBV-positive patients with HCC from healthy individuals. MiR-375 causes G1 stall and death while inhibiting the development and invasion of tumor cells in the liver. The miR-375 transduction cascade has astrocyte up-regulated gene-1 (AEG-1) as a subsequent primary receptor. AEG-1 was discovered to be elevated and is a key oncogene in malignancies of the liver. In initial HCCs, the transcript concentrations of miR-375 and AEG-1 are adversely associated [34].

Compared to normal tissues, the circulating degree of G protein signaling regulator 2-messenger RNA is noticeably increased in malignancies of the liver. According to Cox modeling and Kaplan-Meier curves, there is a correlation between an unfavorable outlook and the increase of G protein signal regulator 2 activities. Additionally, G protein signaling regulator 2-messenger RNA may be a standalone predictor of HBV-caused malignancy in the liver. It is anticipated that the G-protein signaling regulator 2 is also the subject of hepatic cancer treatment [35].

1.2. Long non-coding RNAs (lncRNAs) as diagnostic and therapeutic targets for HBV-associated hepatic malignancy

The ability to distinguish between the lncRNA patterns of malignant and healthy hepatocytes has been made easier by the creation of lncRNA microarrays. Numerous lncRNAs have been the subject of extensive research recently due to their demonstrated carcinogenic as well as tumor-suppressive functions [36]. In other words, because lncRNAs play a crucial regulatory role in important physiological processes, they may be used as potential therapeutic targets as well as diagnostic signals [37]. It is currently known that

many lncRNAs have distinct expression patterns in the majority of malignancy types, especially HBV-accused HCC [38].

Currently, lncRNAs, novel RNA types, and epigenetic determinants have become important players in HBV-caused HCC, impacting the disease's onset, development, assault, and dissemination [39]. On the other hand, by focusing on and modifying the expression of subsequent genes in cancer-related signaling networks, lncRNAs can stimulate the autophagy, expansion, and migration of malignant hepatocytes. These transcripts also alter the function and expression of different cancer suppressors and cancer-causing genes [40].

HBV replication and cancer formation have been linked to a variety of lncRNAs, particularly those that are dysregulated in HBV-associated HCC. The HBV X protein regulates the majority of these dysregulated long noncoding RNAs. Certain lncRNAs' regulatory roles in HBV replication and cancer development were recently identified [41]. In HBV-caused HCC, certain dysregulated lncRNAs may develop into biological signals for rapid identification and drug targets for the disease [42].

In other words, it has been demonstrated that these host-derived long noncoding RNAs, which are frequently dysregulated as a result of viral infection, can act as signals, decoys, guides, or scaffolds to modify and modulate the expression of genes at the transcriptional, post-transcriptional, epigenetic, and even post-translational stages. On the other hand, long non-coding RNAs (lncRNAs) that are found in the nucleus are thought to regulate at the chromatin and transcriptional phases, while those that are found in the cytoplasm are thought to regulate at the post-transcriptional level. LncRNAs may recognize miRNAs using these stages to build lncRNA and miRNA coalitions that control treatment resistance, cancer metastases, activation of cancer cells, and the malignant milieu (Fig. 1) [43].

They are also taking an active role in the biological and pathophysiological mechanisms of HBV-induced HCC. Recent research has indicated that lncRNAs play a critical role in the development and spread of HCC. Via their attachment with DNA, RNA, or protein molecules, or their encoding of small amino acids, HCC-associated lncRNAs have been demonstrated to display abnormal expression and contribute to malignant characteristics such as ongoing growth, bypassing apoptosis, increased vessel creation, and the acquisition of intrusive capability) [44].

Furthermore, the intricate interactions between lncRNA and other biomolecules, including RNA-binding proteins and DNA-binding proteins, are the molecular mechanisms behind lncRNA activity [45]. Therefore, a better comprehension of lncRNA dysregulation might offer fresh perspectives on the etiology of HCC as well as innovative methods for the early detection and management of HCC [46].

The linear lncRNAs are very diverse, which is congruent with their varied biosynthesis and seems to reflect their important regulatory roles in cancerogenesis pathways. This means that linear lncRNAs, also known as guide lncRNAs, can direct the ejection of a material into the right target cell by first attaching to specific amino acids on the cellular surface [47]. Additionally, they may attach and aid in the destruction of particular amino acids or other types of RNA by acting as molecular decoys (also known as decoy lncRNAs). Moreover, they can act as scaffolds (scaffolds lncRNAs), which provide an essential foundation for assembling various molecular elements, such as peptides, as well as other RNAs. Finally, they can control the production of targeted genes in a tissue-specific manner through interactions with transcription variables via chromatin-modifying enzymes [48].

1.3. LncRNAs as decoy molecules

LncRNA frequently functions as a ruse by obstructing a specific molecular route. Following transcription, lncRNA attaches itself immediately to certain protein molecules, like transcription controllers or chromosomal folding peptides, and reduces their ability to operate. LncRNAs attach to transcription controllers directly, preventing transcription elements from doing their jobs and thus inhibiting the transcription of downstream genes. By splashing miRNAs, lncRNAs may influence the expression of the target genes. Target gene expression is upregulated when lncRNAs attach directly to miRNA molecules in particular malignant cells and tissues, blocking them from binding to the target mRNAs [49,50].

1.4. LncRNAs as guide molecules

By helping particular proteins achieve their target site and carry out their physiological tasks, lncRNAs can operate as guiding molecules. Transcription factors are guiding molecules that are found in certain DNA sequences and govern the transcription of genes. Transcription factors interact with lncRNAs frequently [51]. Furthermore, chromatin-modifying proteins can be guided to specific

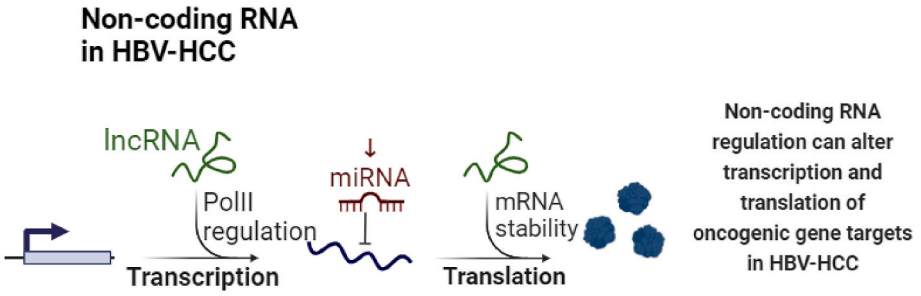

Fig. 1. Regulation of lncRNA in HBV-HCC carcinogenesis.

genomic areas by LncRNAs. Gene expression patterns may alter as a result of these specific epigenetic changes made possible by this targeted recruiting [52].

1.5. LncRNAs as scaffold molecules

LncRNA can function as a key platform, promoting interactions between various molecules and proteins. Moreover, lncRNAs' scaffolding qualities facilitate the development of various macromolecular complexes, which encourages the overlapping and integration of data across many signaling networks [53]. This role also helps to organize and coordinate a variety of cellular functions, such as controlling genetic variation and signal transduction [54].

1.6. LncRNAs as signals

In response to external triggers, signal lncRNAs are expressed in a particular location and at a given time in the cell. This model contains both regulatory and by-product lncRNAs, and transcription can be regulated during initiation, elongation, and cessation. By preventing their target genes from being transcribed or by causing heterochromatin to develop, signal lncRNAs have been shown to cooperate with chromatin-modifying molecules like histone methyltransferases to mute their target genes [55,56]. Moreover, the primary roles of these lncRNAs are to inhibit tumor suppressors and/or increase oncogene expression and HBV replication. It has been discovered that barely any lncRNAs inhibit the growth of tumors, and these are frequently inhibited in HCC [57].

LncRNAs may have a variety of effects on the likelihood of HBV-associated HCC. Therefore, one useful method for examining the biological basis of HCC caused by HBV is to look into lncRNAs that are erratically displayed in HBV-associated hepatic cancer. The inaugural lncRNA to be found to be selectively elevated in hepatic malignancy was HULC (highly up-regulated in liver cancer) [36,58]. In hepatic tumors and lineages of cells, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) production is markedly elevated. This carcinogen suppresses HCC cell death and encourages HCC growth and spread. Mutating MALAT1 causes less tumor cell expansion, infiltration, movement, and apoptosis [59].

Through different mechanisms, MALAT1 contributes to both cell death and hepatic cancer cell growth. MALAT1 is up-regulated in the expansion of malignant hepatocytes and down-regulated in the lysosomal and malignant death of cells [60].

It has been stated that tumor tissues and lineages of cells exhibit more HOTAIR (hox transcript antisense RNA). Further studies have revealed that increased HOTAIR overexpression suppresses the RNA-binding motif protein. Hence, promotes the spread and infiltration of cancer cells with hepatic malignancy. Moreover, by increasing the synthesis of the autophagy-related 3 (ATG3) and autophagy-related 7 (ATG7), HOTAIR upregulation encourages autophagy process. ATG genes are essential for the development of autophagosomes. ATG3 and ATG7 can also influence autophagy-based pathogenic operations like the development and spread of hepatic malignancy (Fig. 2) [61,62].

When HOTAIR is inhibited, malignancy cell survival, relocation, assault, and carcinogenesis are all repressed. According to mechanistic investigations, HOTAIR plays a role in the attraction of macrophages and suppressor cells derived from myeloid precursors to the tumor microcosm as well as in the promotion of malignancy via silencing miR-1 [63,64].

According to the functional study of lncRNAs, lncRNA-H19 expression was elevated in liver cancer stem cells, liver tissue, and the plasma of patients with HCC. However, it was significantly reduced following an incomplete or full treatment response. Less lncRNA-H19 was present in those individuals who had HCC throughout the follow-up. Hence, LncRNA-H19 may be a novel HBV-HCC sensor

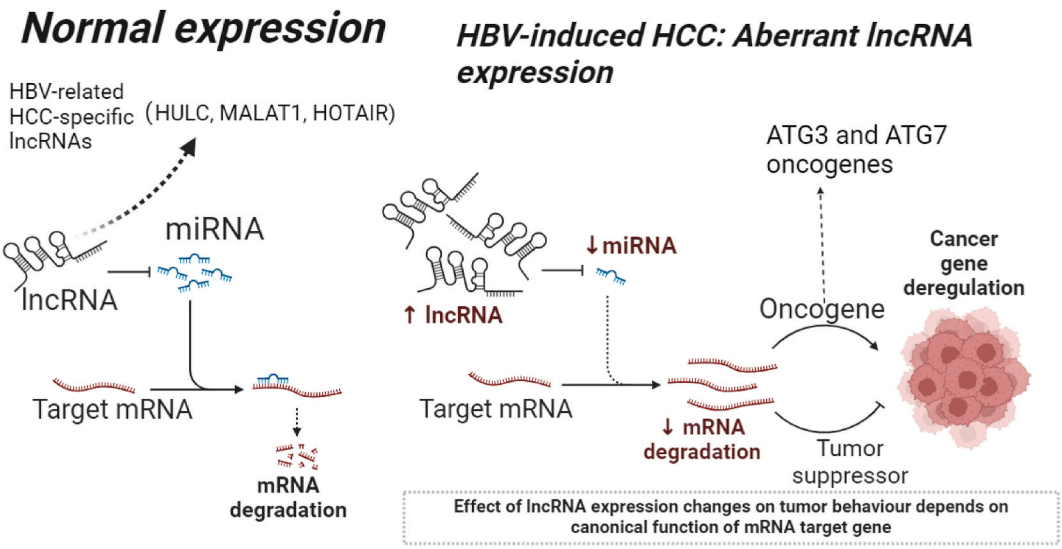

Fig. 2. Normal and abnormal gene expression of lncRNA-miRNA in HBV-associated hepatic malignancy.

[65]. In addition, in HCC tissues and lineages of cells, lncRNA small nucleolar RNA host gene 17 (SNHG17) is significantly elevated and linked to big tumor growth, inadequate transformation, and vascular metastasis [66].

In functional cancer research, it has been established that patients with elevated lncRNAs for urothelial carcinoma associated 1 (UCA1) have a poorer chance of survival and high-grade tumors in HBV-HCC. UCA1 is a malignant lncRNA. Key biological events associated with the advancement of HBV-caused hepatic malignancy, such as the proliferation, encroachment, relocation, metastasis, and vasculature of the malignant hepatocytes, are regulated by UCA1 [67]. Furthermore, according to an operational study, it has been demonstrated that the CCAAT enhancer binding protein alpha (CEBPA) gene's divergent transcript, CEBPA-DT, is involved in HCC. In human HCC tissues with surgical metastatic disease, CEBPA-DT was elevated, and this was directly linked to the poorer prognosis for individuals with HCC [68]. CEBPA-DT increased HCC metastasis by activating Snail1 through DDR2 (discoidin domain-containing receptor 2)/β-catenin-driven reactions with hnRNPC (heterogeneous nuclear ribonucleoprotein C). This suggests that the CEBPA-DT-hnRNPC-DDR2/β-catenin path could be an interesting target for the management of HCC [69].

The anti-tumor immune reaction is dampened in vitro by lncRNA TUC339, which is abundantly expressed in exosomes produced from HCC and promotes tumor development and dissemination in addition to macrophage control of M1/M2 polarization. Microarray results showed that TUC339-induced FcR-mediated scavenging mechanisms and Toll-like receptor (TLR) pathways were down-regulated. Remarkably, TUC339 silencing alters cytokine and chemokine cascades and boosts macrophage phagocytic function [70].

However, there are a few lncRNAs that are markedly down-regulated in HCC. Down-regulated in HCC, MEG3 (maternally expressed 3 genes) is a tumor-suppressive long noncoding RNA. It contributes to apoptosis promotion and proliferation of cell inhibition. There has been evidence of decreased MEG3 expression in the setting of HBV-related HCC. Another lncRNA that has been discovered to be down-regulated in cancer cells is lncRNA-p21. Tumor development and enhanced cell growth are linked to its downregulation. LncRNA-p21 can impede the growth of HCC by interacting with several proteins and signaling pathways [39].

However, lncRNAs SNHG8 (small nucleolar RNA host genes 8), lncRNAW42, LINC01225, phosphatidylinositol transfer protein alpha antisense RNA 1 (PITPNA-AS1), and zinc finger e-box binding homeobox 1 antisense RNA 1 (ZEB1-AS1) demonstrate oncogenic properties by causing the proliferation and start of metastatic dissemination of HCC cells [71–74].

Moreover, in HCC, LINC01977 (long intergenic non-protein coding RNA 1977) is also substantially expressed. In those suffering from HCC, greater LINC01977 concentrations are associated with a worse survival rate. According to scientific research, LINC01977 stimulated the development and dissemination of HCC both in tissue and in the laboratory. Scientifically, LINC01977 directly attaches to RNA-binding motif protein 39 (RBM39) to encourage neurogenic locus notch homolog protein 2 (notch2) entrances into the cell's nucleus and halt notch2 degradation and destruction [75]. Moreover, the long-term viability of LINC01977 is increased by the RNA-attaching molecule human insulin-like growth factor 2 (IGF2) mRNA binding proteins 2 (IGF2BP2), one of the n6-methyladenosine (m6A) alteration readers, which led to its significant presence in HCC. As a result, the information points to LINC01977's interaction with RBM39 and its possible application as a sensor and treatment option for cancer patients with HCC by blocking notch2 folding and destruction (Table 1) [76]. By interfacing alongside the miR-655-3p/SMAD5 (mothers against decapentaplegic homolog 5) axis, LINC01194 is also increased in the HCC lineage and regulates the growth and movement of HCC cells, offering novel markers for HCC detection and therapy [77].

Tumor growth, HBV infection, and HBx quantity are related to LINC00152 expression. The overall survival rate is likewise reduced in the presence of increased LINC00152 expression. The HBx protein up-regulates LINC00152, which promotes carcinogenesis in vivo as well as the growth and EMT of lineages of HCC cells in vitro. It has been demonstrated that LINC00152 activates the mammalian/mechanistic target of the rapamycin (mTOR) pathway, a well-known dysregulated system implicated in the etiology of HCC [78].

Table 1
Various miRNA expressions in HBV-caused hepatic malignancy.

Types of micro RNAs	their source	Different kinds of molecular techniques for their detection and quantification	Their target oncogenes	Ref.
miR-335-5p, miR-172-5p, and miR-1285-5p	Serum	qRT-PCR	Up-regulated	[79]
miR-636 and miR-187-5p	Serum	qRT-PCR	Up-regulated	[17]
circRNA1002	Serum	qRT-PCR	Up-regulated	[20]
miRNA-21	Serum	qRT-PCR	Up-regulated	[22]
miR-324-3p	Serum	qRT-PCR	Up-regulated	[24]
miR-210-3p	Serum	qRT-PCR	down-regulated	[25]
miR-497-5p	Serum	qRT-PCR (via reverse transcription)	Down-regulated	[16]
miR-3	Serum	qRT-PCR	Down-regulated	[27]
miR-718	Serum	qRT-PCR	Down-regulated	[29]
miR-122	Serum	qRT-PCR (via reverse transcription) and western blot	Down-regulated	[31]
miR-522	Serum	qRT-PCR	Down-regulated	[32]
miR-0308-3p	Hepatic-biopsy	qRT-PCR	Down-regulated	[33]
miR-375	Serum	qRT-PCR and solexa sequencing	Up-regulated	[34]
miR-223p	Serum	Nano-string quantitative assay and SYBR green qRT-PCR	Down-regulated	[19]
miR-1	Serum	qRT-PCR	Up-regulated	[64]

HBV = hepatitis B virus; RNAs = ribonucleic acids; miR = microRNA; circRNA = circulating ribonucleic acid; qRT-PCR = quantitative real time polymerase chain reaction; SYBR green = a dsDNA coupling dye may be applied to measure the volume of primers produced throughout a PCR by monitoring the total generated fluorescence.

1.7. Extracellular vesicles (EVs) as diagnostic and therapeutic targets for HBV-associated hepatic malignancy

EVs are enclosed lipid nanoscale capsules released by several kinds of cells. As extracellular vesicles (EVs) take part in communication between cells, they are diverse and actual actors in liver function and disease. Liver cancer growth and advancement are linked to extracellular vesicles (EVs) that contain active payloads composed of proteins, lipids, and genomes [80]. EVs have come to light as intriguing contenders for prospective markers. Due to their versatility in eliciting cell-free responses to therapy and their

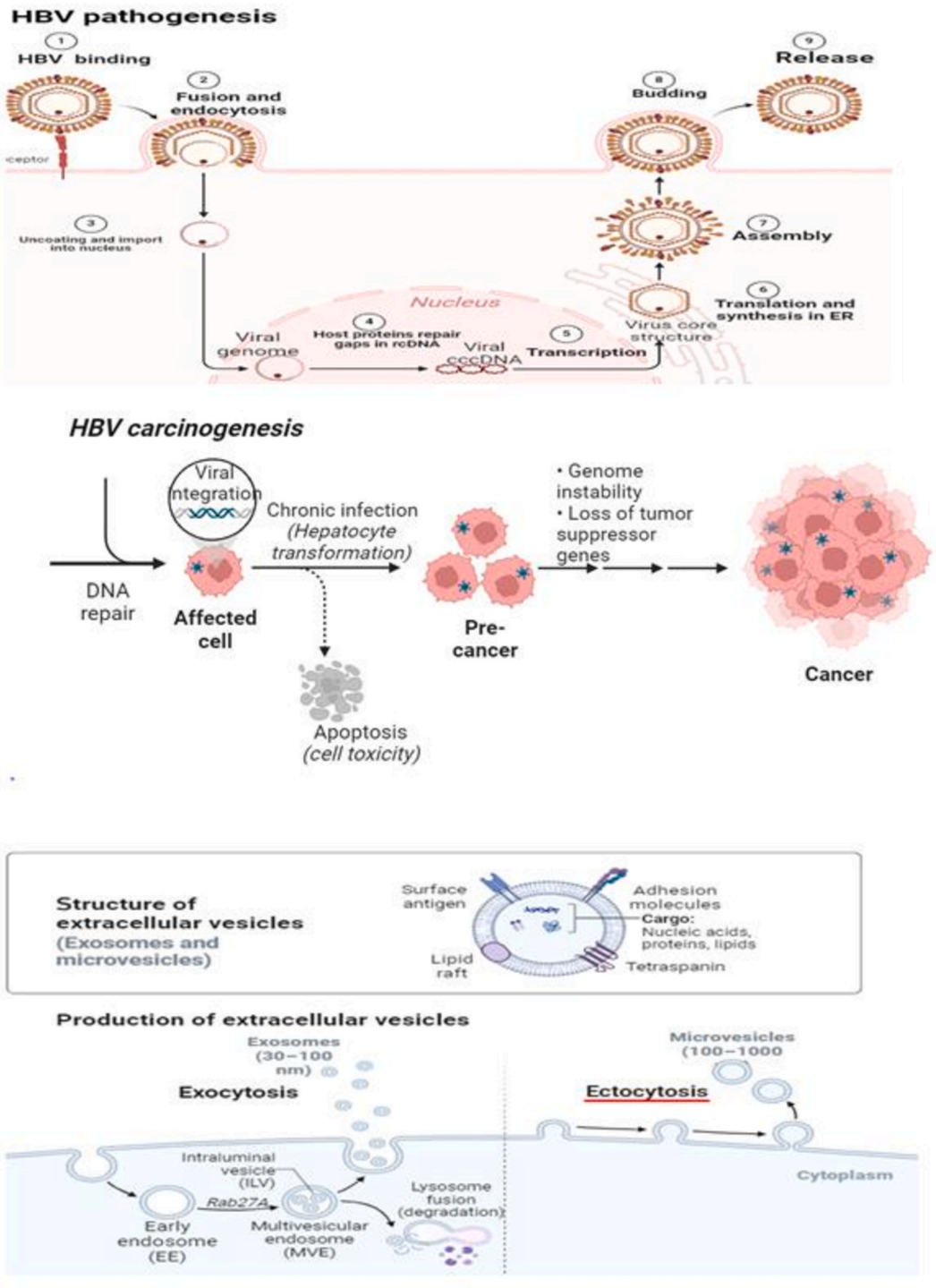

Fig. 3. The generation of Extracellular Vesicles (EVs) from HBV-infected hepatocytes.

payload capacity, EVs can be utilized as innovative indicators for HBV-induced hepatic malignancy. EVs have garnered substantial interest from scientists in the past 20 years as fluid biopsy technology has advanced and become more clinically used [81].

Since EVs are found in a range of cellular secretions and contain biologically active substances derived from initial cells. They are also highly valuable as prognostic as well as diagnostic indicators of HBV-caused hepatic malignancy. EVs are also being studied as possible targets for treatment and delivery systems for pharmaceuticals. EVs generated by different cells can be released and absorbed by malignant hepatocytes. Previous research suggests that liver EVs are also crucial in the pathophysiology of HBV-induced liver cancer [82,83].

According to previous research, EVs were essential mediators that play a role in carcinogenesis, angiogenesis, immunology, and the spread of hepatic cancer cells among various facets of liver tumor pathophysiology. The functions of EVs in the development of HCC and tumor progression are being thoroughly studied. EVs seem to be secreted more by malignant hepatocytes than non-malignant cells [84].

HCC cell lines secrete a higher quantity of EVs, and these EVs have pro-metastatic impacts. Following treatment with anticancer agents, HCC cellular models generated a greater quantity of EVs, which may have triggered the natural killer cell response to provoke anticancer immune responses. EVs produced from HCC cells known as Hep3B or human hepatoma cell line decrease the activity of TAK1 (transformational differentiation factor β activated kinase-1), an important controller that governs cell balance and cancer in the liver. Simultaneously, recipient cells experience changes in the TAK1-related subsequent pathways. HCC cell-derived EVs stimulate the development and infiltration of the original HCC cells in addition to delivering miRNAs into recipient cells [82].

Furthermore, distribution of size investigation, morphological examination, and membrane biomarker detection were used to corroborate the purification of single-cell EVs. Using satellite spectrometry-based label-free estimation, it was possible to identify 61 and 63 genes that were differently displayed in the plasma EVs of individuals with liver cancer and liver cirrhosis, respectively ($p < 0.05$) [83].

The isolation of EVs from individuals with malignancies of the liver included LC3B (microtubule-associated proteins 1A/1B light chain 3B) + EVs that expressed attachment molecules from epidermal cancer cells, suggesting that these LC3B + EVs were derived from epidermal malignant cells. Compared with those with non-malignant liver disease and normal controls, hepatic cancer patients had a dramatically greater amount of serum LC3B + EVs and HSP90α (heat shock protein 90α) + LC3B + EVs. This indicates that serum LC3B + EVs harboring biologically active substances can be isolated and detected as an efficient detection signal for malignancies of the liver. They may also be employed as an option for immunological surveillance and clinical outlook forecasting [85].

EVs are resilient to enzymes like RNases and proteases, easily absorbed, and biodegradable. Due to these qualities, EVs are perfect for delivering substances, including proteins, medicines, microRNAs, and silencing RNA [86].

Hepatocytes may be stimulated to release more EVs by HBV infection. It has been noted and researched that HBV infected hepatic cells can produce EVs [87]. The process by which HBV-infected cells produce EVs is mostly dependent on exocytosis. Exocytosis plays a role in the generation and release of EVs carrying viral components during HBV infection [88]. Hepatocytes are the site of HBV replication during viral infection. Within these intracellular spaces, known as endosomes, the virus assembles its particles. After exocytosis, these endosomes merge with the plasma membrane to release EVs that contain viral components [89].

There are several ways in which HBV-derived material might be included in electric vehicles. Initially, the produced viral particles can be directly encapsulated into late endosomes or multivesicular bodies (MVBs) to form intraluminal vesicles (ILVs), which are released during exocytosis when they fuse with the plasma membrane. Then, HBV replication-derived proteins and nucleic acids can be segregated into ILVs before being packaged into EVs [90,91].

Following HBV infection, the release of EVs from infected cells through exocytosis serves multiple functions. In the liver microenvironment, it first permits the intercellular transfer of infectious virions to adjacent uninfected hepatocytes or other vulnerable cell types. The establishment of chronic infection and the dissemination of the virus can both be facilitated by the transmission of functional viral particles through EV-mediated pathways [92]. Ectocytosis is another critical process that involves the formation of EVs in HBV-liver cells. EVs can be continuously released by infected hepatocytes via ectocytosis. These generated EVs have important effects on immunological evasion, cellular growth, and viral transmission (Fig. 3) [93].

The content of these released EVs can also be changed by these inflammatory cytokines, increasing their immunological activity and capacity to elicit immune responses [94]. It is unclear exactly how HBV-mediated EV release regulation affects viral pathogenesis. On the other hand, it is thought that by promoting viral dissemination and attenuating antiviral immune reactions, these produced EVs could aid in immune evasion. They might also be prospective targets for therapeutic interventions or biomarkers for tracking the course of a disease [95].

Generally, interactions with host cellular machinery and inflammatory signaling pathways cause HBV infection to increase the release of EVs from infected hepatocytes. To completely comprehend the consequences of this modification on viral pathogenesis and its possible therapeutic uses, more investigation is required [96]. Although many possible mechanisms have been suggested, the complex pathways and signaling cascades involved in HBV-mediated control of EV release remain unclear. Some of the complex signaling cascades and pathways involved in HBV-mediated EV release modulation are discussed below:

Activation of cellular pathways: Cellular signaling pathways implicated in extracellular volatile organic compounds (EV) emission can be activated by HBV infection. For instance, EV biogenesis and secretion have been linked to the mitogen-activated protein kinase (MAPK) pathway, which includes extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), and p38 MAPK. The stimulation of these mechanisms by HBV may improve EV release [97]. In addition, host elements such Rab GTPases and ESCRT complexes that are involved in vesicle generation and secretion processes help control EV release during HBV infection [98].

Viral proteins: The HBV virus encodes several viral proteins that may affect EV release either directly or indirectly. For example, it has been demonstrated that the HBx protein stimulates EV secretion by activating the exocyst complex and eliciting Rab27A

expression. Furthermore, to control EV synthesis, the viral envelope protein may interact with components of the host cell involved in intracellular vesicle trafficking [99,100].

Immune response modulation: Hepatitis B virus infection modifies the immune response, which may affect the release of extracellular vesicles (EVs) from infected cells. It has been demonstrated that EV secretion processes are regulated by inflammatory cytokines, such as interferons and tumor necrosis factor-alpha (TNF-α), via several signaling pathways [101].

Cellular stress response: Heat shock proteins or endoplasmic reticulum (ER) stress-related pathways may be how cellular stress responses brought on by HBV infection affect EV release processes, either directly or indirectly [102].

Exosomal cargo modification: An additional feature of HBV-mediated EV regulation is the modification of EVs' cargo composition following viral infection. Viral proteins or nucleic acids that are specifically packaged into exosomes may change how those exosomes behave when they get to the recipient cells [103].

Knowing the complex mechanisms and signaling cascades involved in HBV-mediated control of EV release may help to clarify pathophysiology, the virus's methods of immune evasion, and possible targets for therapeutic intervention in the treatment of hepatitis B infections or associated illnesses [104]. Moreover, it's critical to comprehend the regulatory processes underlying HBV-induced EV release to create novel therapeutic approaches to combat this enduring viral infection. By altering these systems, it may be possible to stop the virus from replicating and strengthen the body's defenses against HBV [105]. On the other hand, clarifying the regulatory mechanisms controlling HBV-induced EV release is a crucial field of study with ramifications for comprehending the pathophysiology of viruses and creating cutting-edge treatments to combat hepatitis B virus infection [106].

Malignant hepatocytes can also generate exosomes and microvesicles, which are extracellular vesicles that can move biologically active RNAs and protein molecules throughout cells [107].

Hepatocytes with disrupted miRNA apparatus had increased HBV replication, suggesting an innate miRNA-mediated antiviral state. There may be a connection between HBV replication and EV-related miRNA as disruption with EV release significantly reduced HBV replication in the presence of usual miRNA synthesis. miRNA synthesis in EVs was altered by HBV replication, according to microarray and qPCR analyses [108].

Adjacent hepatocytes can get the viral genome through EVs generated from virally infected hepatocytes. EVs harboring the DNA of the hepatitis virus are also discharged into the circulation. HBV DNA is present in serum EVs obtained from individuals who have a persistent hepatitis B infection and may infect healthy hepatocytes [109]. EVs transfer the viral payload to the target cells while shielding the viral DNA from immunological reactions. Consequently, blocking EV discharge from HBV-infected hepatocytes may lessen the spread of HBV [110].

EVs play vital functions as intratumoral mediators in the genesis and advancement of HCC. Initially, EVs are used by HCC to modify hepatocytes. Healthy hepatocytes' relocation, survival, and multiplication are enhanced by HCC cells' delivery of EVs that include protumorigenic RNAs and peptides along with lncRNA remodeling regulators [111].

EVs are another way that HCC cells communicate with one another within the HCC community. By focusing on PTEN, tumor-derived EVs promote the growth of HCC cells and hence accelerate the creation of tumors [112]. EVs produced by HCC may be separated from cultured cells and absorbed by nearby cells. Evs exhibit a significant alteration in the expression of many ultra-conserved long noncoding RNAs (ucRNA) as contrasted with donor cells. Cloning and identification of a 1198-bp ucRNA known as TUC339 revealed that it is the most highly substantially expressed ucRNA in Evs produced from HCC cells [113].

TUC339 has been biologically linked to the regulation of attachment and the development of malignant cells. Proliferation in soft agar, clonogenic expansion, and HCC cell growth are all decreased when TUC339 is suppressed by siRNA. Consequently, TUC339 intercellular transfer is a distinct signaling pathway that tumor cells can use to encourage the growth and dissemination of HCC [114]. The possible functions of ucRNA in HCC provide evidence for the presence of specific pathways, governing lncRNA export from cells and link lncRNA trafficking via EVs to tumor cells' ability to regulate their local biological milieu. Cells inside the microenvironment can impact one another genetically through the intercellular exchange of functionally active RNA particles via EVs [115].

HCC growth is made worse by interactions between tumor cells and the tumor microenvironment via EVs. In addition, by conveying the lncRNA TUC339, HCC-derived EVs polarize macrophages to an M2 phenotype, analogous to tumor-related macrophages. By producing EVs, HCC cells also hinder $CD8^+$ T cells' ability to fight tumors [70,116].

Apart from their functions in intratumoral interactions, EVs originating from extrahepatic cells have an impact on the growth and advancement of HCC. EVs allow various malignant cell types to communicate with one another through biological information. EVs also promote HCC dissemination. Extremely invasive HCCs release extracellular vesicles (EVs) that trigger mesenchymal-epithelial transition (EMT) in low-metastatic tumor cells through mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) signaling [117]. Because they boost growth and lessen DNA damage, EVs secreted from fatty tissue aid in the evolution of HCC. On the other hand, fatty tissue gets EVs from HCC cells, which cause them to change into pro-tumorigenic, pro-inflammatory and pro-angiogenic profiles [118]. In addition, it is well recognized that HBV affects several hepatocyte pathways and cell cycle regulation, which leads to the development of HCC [119].

1.8. Circulating cell free DNA (cfDNA) as diagnostic and therapeutic targets for HBV-associated hepatic malignancy

Two benign and exciting ways to measure the amount of DNA in the circulatory system are circulating cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA). ctDNA is another name for the cfDNA obtained from hepatic malignant cells. When combined with additional circulating biomarkers and are referred to as fluid biopsies. Over 75 % of advanced stages of hepatic malignancy have detectable cfDNA. Furthermore, hepatic malignancy burden is reflected in the concentration of ctDNA, which can reach up to 40 % in late-stage illness and 1 % in cancer in its early stages. Keep in mind that whereas ctDNA pertains to the particular portion of cfDNA that

bears certain molecular changes, cfDNA pertains to the material used in the sample of experiments [120–122].

As a non-invasive method of following the development of genetic changes unique to a hepatic malignancy, cfDNA is now working its way into clinical research. Furthermore, notably in the malignant context, cfDNA is showing potential as a proxy for evaluating the molecular composition of tumors and mitigating the sampling prejudices brought on by intra-tumor genetic variability [123].

It is expected that the identification and evaluation of cfDNA will get more precise and accurate with advancements in 'genomics and molecular biology approaches, as well as a better comprehension of the molecular mechanisms of cancer. This will allow cfDNA evaluation to be employed as a diagnostic tool for individuals with the initial stages of cancer [124]. Apoptosis, tissue necrosis, and excretion produce cfDNA, which is linked to hepatic malignancy and its microcosms through genomic and epigenetic characteristics. Suggested therapeutic uses for the profiling of genetic and epigenetic changes in cfDNA include early illness identification, therapeutic response foresight, and real-time prediction [125].

Tumor-related signals such as mutations and aberrant epigenetic alterations like 5-hydroxymethylcytosine and 5-methylcytosine can be found through the analysis of serum-based cfDNA. In contrast to genetic alterations, epigenetic modifications are recoverable and have a greater impact on gene expression [126]. Epigenetic modifications of gene transcription are transmissible and do not involve modifications to the DNA structure directly. Therefore, research on epigenetic control and the underlying molecular mechanisms is making a significant contribution to our understanding of the molecular processes behind the diversity and progression of hepatic cancer. Furthermore, this information might be useful in identifying biomarkers for the identification and outlook of liver cancer, as well as novel targets for subsequent therapies that will be more effective [127].

A significant amount of DNA is discharged into the bloodstream by dead cells, immunological opposition, numerous organ dysfunctions, and hepatic cancer brought on by HBV. The malignant microenvironment's apoptosis and immunological system-killed cells are probably the causes of the increased cfDNA concentrations observed in those with hepatic cancer. Less than 0.01 % of the entire spectrum of cfDNA is thought to consist of ctDNA. Nevertheless, patient-derived cfDNA might include tumor-associated genomic and epigenetic details such as somatic mutations and cytosine alterations like DNA methylation and hydroxymethylation, and these provide the availability of ctDNA [128–130].

An innovative, non-invasive method for identifying genetic changes in cancer is ctDNA. Furthermore, HBV-induced hepatic malignancy is being identified by copy number variants (CNVs) in cfDNA as noninvasive signals. cfDNA CNVs identified by low-coverage whole-genome sequencing (WGS) may have possible applications as predictive sensors for individuals with HCC. Specifically, the multiple-level CNVs have a significant correlation with both overall survival (OS) and recurrence-free survival (RFS) in individuals with HCC undergoing revolutionary therapies [12]. Moreover, the characteristics of cfDNA cleavage length and end motifs, which arise from distinct nucleosome assembly processes and CNVs between normal and tumorous tissues, are combined to form the cfDNA fragmentome [131]. On the other hand, the intratumor diversity in multinodular HCC is captured by genetic evaluation of cfDNA, underscoring the prospect of cfDNA as a powerful and noninvasive means of targeted therapy, and the end-motif arrangement, fragment size choice, and CNV signals in cfDNA are useful tools for diagnosing HBV-caused HCC patients [132,133]. Previous study has revealed the critical role minimal residual disease (MRD) serves in tumor spread and relapse. MRD is thought to play a significant role in both causing and encouraging postoperative reappearance [134]. Determining MRD is essential for determining the long-term prognosis and directing the selection of adjuvant therapy in HBV-HCC [135,136].

Research indicates that after the tumor is surgically eliminated, cfDNA returns to normal. Primarily remaining malignant cells generated postoperative cfDNA. When postoperative cfDNA concentrations remain elevated following surgery, it suggests the presence of MRD that is not detectable with preoperative imaging examinations [137]. Studies show that cfDNA returns to normal following surgical excision of the tumor. Most of the cancerous cells that were left behind after surgery produced cfDNA. Postoperative cfDNA levels that stay high after surgery indicate the possibility of MRD that is not picked up by preoperative imaging tests [138,139]. Since ctDNA can be measured at every phase of HCC development, it is possible to identify changes in the main controlling loci of hepatic tumorigenesis. Because of these characteristics, ctDNA is a promising prospective indicator for the prognosis as well as the diagnosis of HBV-associated hepatic cancer [140].

Finding aberrant forms of cfDNA derived from malignant cells ctDNA offers a new way to track disease progression and identify malignancy. When DNA breaks free from cancerous cells, cancer-specific genetic as well as epigenetic modifications are transferred to ctDNA, which is not present in healthy cfDNA [141]. This aids in separating ctDNA from cfDNA. Malignancies containing ctDNA level, genetic integrity, mutations in genes, methylation of DNA changes, and gene pairings exhibit notable changes in their genomic and epigenetic characteristics. A number of factors, like the kind of molecular change that commonly affects genes, may vary even within a single individual or group. For instance, the most often mutated genes are at-rich interactive domain-containing protein 1A (ARID1A), ataxia-telangiesctasia mutated (ATM), catenin beta 1 (CTNNB1), telomerase reverse transcriptase (TERT), and tumor protein p53 (TP53), with their mutation rate of 7–13 %, 25–39 %, 17–42 %, 42–69 %, and 32–80 % respectively. However, the most frequently expanded genes are mesenchymal epithelial transition (MET) and Cyclin D1 (CCND) [142,143].

Methylation investigations of circulating cancer DNA have demonstrated encouraging results for early diagnosis of HCC in people who are at risk. For HBV-nduced hepatic malignancy monitoring, methylation markers in cell free serum DNA provides a novel option [144]. In other words, the natural progression of HBV-HCC development aligned with the dynamic characteristics of cfDNA methylation [145]. In addition, a repeated evaluation of the mutational landscape and cfDNA levels can also reveal dynamic qualitative and quantitative alterations in the cfDNA following medication.

In the past few years, DNA methylation such as 5-methylcytosine and 5-hydroxymethylcytosine have been investigated as potential indicator approaches in cfDNA for many malignancies particularly HCC, as epigenetic alterations play a crucial role in the control of genes and pathophysiology [146]. In patients with HBV-induced hepatic cancer, significant methylation processes is frequently happened in TRG5 (T-cell receptor gamma variable 5), TFPI2 (Tissue Factor Pathway Inhibitor 2), RASSF1 (RAS-association domain

family member 1A), and XPO4 (exportin 4) genes compare to those without hepatic cancer [147].

DNA methyltransferases (DNMTs) are the enzymes responsible for the inheritable chemical transformation known as methylation, which takes place on the cytosine ring at location five. The process of DNA methylation primarily influences what are known as CpG locations, which are Cytosine bases (C) next to Guanines (G). As has been shown in the majority of malignancies, notably HCC, methylation in the gene domain increases the expression of genes, but methylation in the gene promoter area generally results in transcriptional inhibition. DNA methylation flaws and the biological process that drives them are strongly linked to hepatic cancer. Hepatic cancer is caused by three different types of changes in DNA methylation, such as worldwide hypomethylation of genes and DNA sequences that results in instability in the genome and carcinogen stimulation, hypermethylation of the CpGIs in promoter areas of malignant inhibitor genes, and abnormal production of DNMTs [148–151].

For the control and progression of the genome, de novo DNA methyltransferases 3A (DNMT3A) and DNMT 3B (de novo DNA methyltransferases 3B) are crucial to methylate DNA at cytosines. This mechanism disruption is linked to several illnesses, especially hepatic carcinoma linked to HBV [152]. Furthermore, an enzyme known as DNMT 1 (DNA methyltransferase 1) is thought to be exclusively engaged in the maintenance methylation process, which is a passive exchange of genomic methylation sequences. Early research reveals that DNMT1 is merely a maintenance methyltransferase but also contributes to the de novo methylation of a small but crucial region of the genome. It reacts to several regulating signals that modulate both its placement and its function (Fig. 4) [153].

In the cfDNA of individuals with HCC with arterial assault, cancer-associated abnormalities that indicated a shortened recurrence-free time were more easily found. Nevertheless, invasion of the vascular system frequently indicates that the malignancy has spread, implying that these cfDNA alterations may not represent the best indicators of initial HCC identification [154]. Furthermore, the restricted number of HCC-associated alterations in this research might assist with clarifying why the heterogeneity of tumors contributed to the low observed identification probability. New hepatic cancer-associated somatic modifications will be found in cfDNA as technology develops, but because of the diversity of tumors and the technological need for deep DNA sequencing, it is still challenging to use cfDNA alterations as a useful tool for initial HCC identification [155].

The peripheral bloodstream is among the most frequently utilized for the extraction of cfDNA, although additional bodily fluids such as urine, saliva, pleural effusion, and cerebrospinal fluid are also useful for separating cfDNA that could represent various kinds of carcinoma of the liver phases. Right now, magnetic bead-based and column-based techniques are the most widely used for cfDNA separation. To increase the specificity and precision of cfDNA alteration identification, the main target molecules in cfDNA analyzing techniques are tumor-specific modification identification, epigenetic identification, and profiling by DNA sequencing. Bisulfite therapy and attraction advancement are common methods for profiling epigenetic modifications in cfDNA. As a result, significant efforts have been undertaken to leverage these cfDNA gene targets to find useful indicators for the early identification of HCC [156–158].

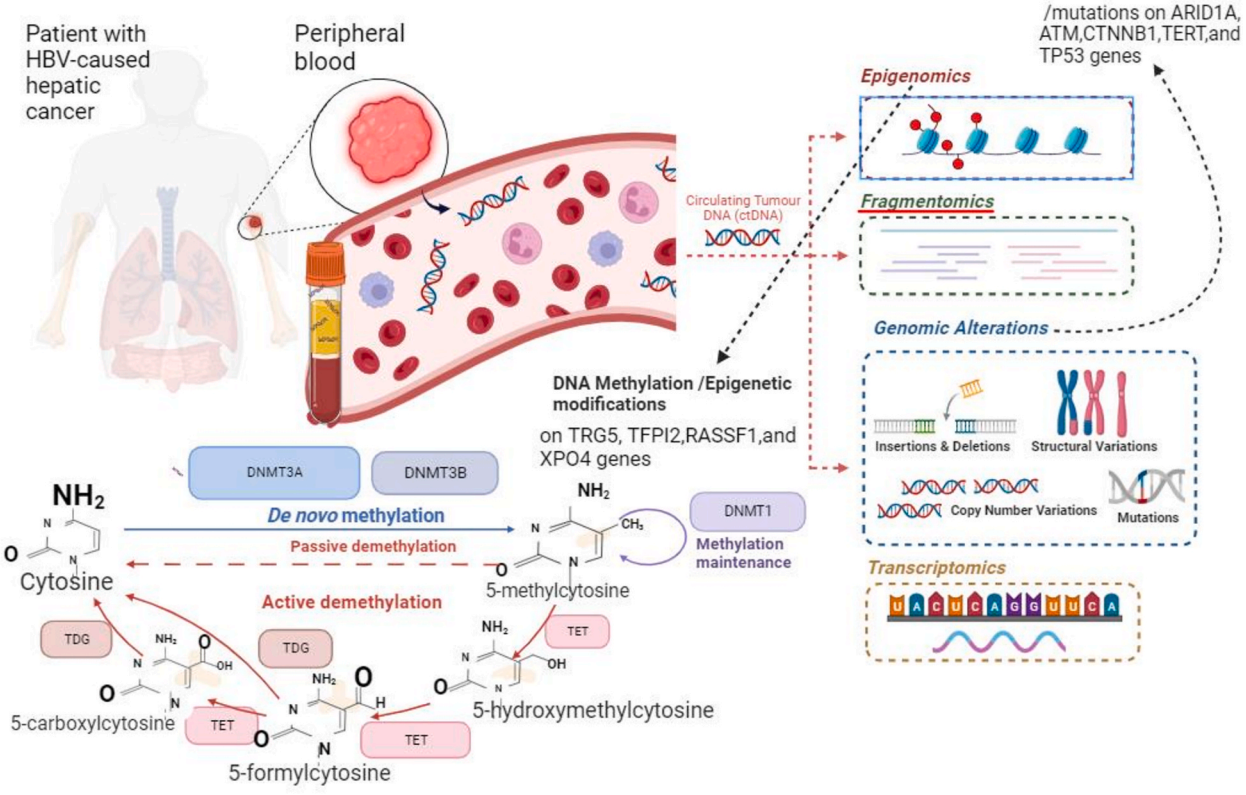

Fig. 4. Epigenetic modification and genetic alteration in HBV-caused hepatic malignancy.

Integrated PCR-based methods and NGS (next-generation sequencing) can measure tumor-specific changes in cfDNA [159].

In general, circulating cfDNA analysis shows a lot of potential as a non-invasive method for identifying hepatic malignancy linked to HBV and directing treatment approaches toward more successful individualized care plans for those suffering from this illness [160]. In addition, comprehending HBV's role in cfDNA release aids in the development of diagnostic instruments that track the dynamics of viral load as the infection progresses and assess the effectiveness of treatments against HBV [161].

2. Conclusions

The current review summarized the diagnostic, therapeutic, and prognostic importance of tumor-derived molecular and genetic signals such as extracellular vesicle microRNAs, extracellular vesicles, long non-coding RNA, and circulating DNA in hepatitis B virus-related hepatic malignancy. Furthermore, these indicators can monitor the expression of oncogenes either by downregulating or upregulating them during HBV-induced hepatocarcinogenesis.

2.1. Recommendations and future perspectives

We recommend academic, scientific, and research communities to perform advanced original molecular research at the molecular and genomic levels to characterize and discover various vital molecular and genetic biomarkers via analyzing the serum and hepatic biopsy of patients with hepatitis B virus -induced hepatic carcinoma. It is also better to analyze large biological datasets using bio-informatics to obtain substantial diagnostic, prognostic, and therapeutic molecular and genetic signals, as well as sensors. On the other hand, current investigations into the use of targeted therapies and molecular and genetic signals as diagnostic tools have created new opportunities for the effective management of HBV-caused hepatic malignancy. By putting these suggestions into practice, patient results will be greatly improved by early diagnosis, customized treatments based on underlying genetic changes, and improved prediction models with multi-omic data analysis techniques leading to personalized medical strategies.

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Adane Adugna: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Yalew Muche:** Writing – review & editing, Writing – original draft, Validation, Supervision. **Abateneh Melkamu:** Writing – original draft, Visualization. **Mohammed Jemal:** Writing – review & editing, Writing – original draft. **Habtamu Belew:** Visualization, Validation. **Gashaw Azanaw:** Writing – review & editing, Writing – original draft, Visualization, Validation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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