Molecular Mechanism Of Hepatitis B Virus: A Pathway From Gene Framework To mRNA Degradation Via Crosstalk With Cell Signaling Network By miR-1, miR-2, miR-3: A Narrative Review.

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ABSTRACT:

Background: The hepatitis B virus (HBV) possesses a circular, partially double-stranded DNA genome of approximately 3.2 kb, organized into four overlapping open reading frames encoding the surface (HBsAg), core (HBcAg), polymerase, and X proteins. Despite its compact genome and extensive gene overlap, HBV maintains exceptional replication fidelity and adaptability. Understanding the molecular organization of its genome, transcriptional regulation, and translational strategies is essential to elucidate how HBV sustains replication and persistence under strong structural constraints.

Methods: A detailed genomic and molecular analysis of HBV architecture, transcriptional units, and translational mechanisms was conducted using published data. Emphasis was placed on the functions of pregenomic RNA (pgRNA), preC-RNA, preS1-RNA, and S-RNA, along with structural features of the core and polymerase proteins and HBV-encoded microRNAs.

Results: HBV utilizes overlapping open reading frames, shared polyadenylation sites, and leaky scanning to maximize protein diversity from limited coding sequences. The pgRNA serves as both mRNA and replication template, while the polymerase domains (terminal protein, reverse transcriptase, RNase H) coordinate reverse transcription. Viral microRNAs contribute to host–virus regulatory interactions, promoting persistence.

Conclusions: HBV exemplifies a highly efficient genetic system that achieves functional complexity within minimal genomic space. Understanding these overlapping mechanisms provides insight into viral replication control and therapeutic target development.

Keywords: Hepatitis B virus; viral genome; pregenomic RNA; HBV polymerase; core antigen; overlapping open reading frames; reverse transcription; viral microRNA; serotypes; genotypes.

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INTRODUCTION:

The viral genetic material consists of a circular, partially double-stranded DNA molecule approximately 3.2 kilobases in length. It contains four overlapping open reading frames (ORFs), each governed by an individual promoter while sharing a common polyadenylation site (1). These ORFs encode

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the surface antigen (HBsAg), core antigen (HBcAg), polymerase, and X (HBxAg) proteins. Owing to the compact genome, viral genes are arranged in overlapping translational frames, with regulatory elements located within the coding regions (2). Considering the limited genome size, extensive overlap of ORFs, and the essential role of nearly every nucleotide, a high mutation rate could potentially compromise viral survival. However, HBV exhibits remarkable adaptability, maintaining replication efficiency and genome stability despite these structural constraints. (3).

The hepatitis B virus (HBV) is classified into four principal serotypes- adr, adw, ayr, and ayw- based on the antigenic variations present on its envelope proteins, and into eight major genotypes designated A through H. Variations among these genotypes influence the severity and progression of the disease, the risk of developing complications, therapeutic response, and potentially, vaccine efficacy (4). Certain genotypes are also associated with distinct transmission routes that predominate in specific geographical regions. During natural HBV infection, an excess of non-infectious subviral particles (SVPs) is produced, which lack the viral nucleocapsid but contain the envelope proteins in their Large (L), Middle (M), and Small (S) forms. These proteins share an identical C-terminal region, whereas M possesses an additional pre-S2 domain, and L includes an extra pre-S1 domain beyond that of M. Substantial evidence indicates that a region within the pre-S1 domain of the L protein interacts with an as-yet-unidentified host receptor during the viral entry process (5).

HBV Genes and Translation of Viral RNA Transcripts into Viral Isoform Proteins:

On the basis of coding capacity of the HBV mRNA based on the genes located on HBV DNA it divided into four categories (6):

1. Pregenomic RNA (pg-RNA, 3.4 kb)
2. preC-RNA, 3.5 kb
3. preS1-RNA, 2.4 kb
4. S-RNA, 2.1 kb

Pregenomic RNA (pg-RNA, 3.4 kb): HBV possesses a small, partially double-stranded DNA genome, from which one of the major transcripts produced is the pregenomic RNA (pgRNA) of approximately 3.4 kilobases. This pgRNA functions dually—acting as the messenger RNA for the synthesis of core and polymerase (P) proteins, and simultaneously serving as the template for reverse transcription during viral replication (6).

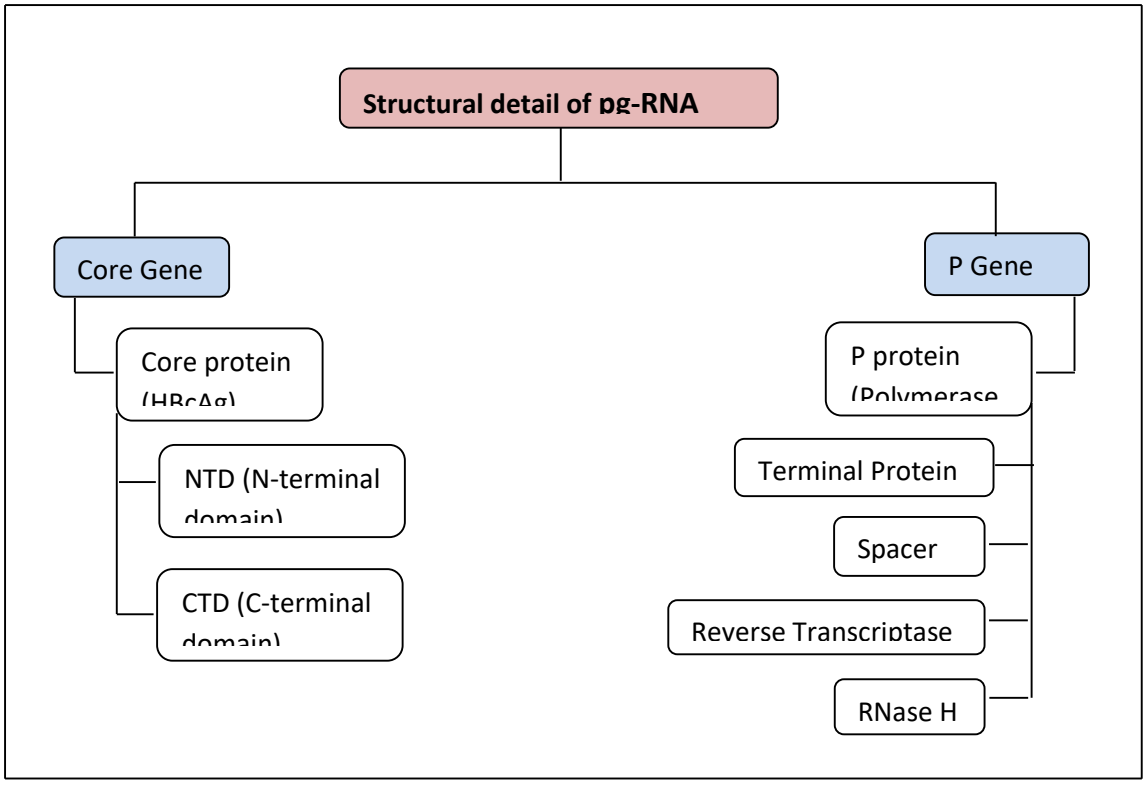

Figure no 1: Showing the structural detail and various domains and subdomain of about pregenomic-RNA.

Structural detail about pg-RNA, 3.4 kb:

The pregenomic RNA (pgRNA) of HBV is bicistronic in nature, meaning it carries coding information for two proteins—the core and polymerase (P) proteins—within overlapping regions of the same RNA molecule. Translation of the polymerase gene occurs through a mechanism known as leaky scanning, in which the ribosome bypasses the first initiation codon (AUG) used for the core protein and initiates translation at the downstream AUG corresponding to the polymerase gene. The pgRNA also contains two conserved direct repeat regions (DR1 and DR2) and terminates with a polyadenylated [poly(A)] tail at the 3′ end. Translation begins from the 5′ capped end of the pgRNA, ultimately producing two major viral proteins: the core antigen (HBcAg) and the polymerase (P) protein (6).

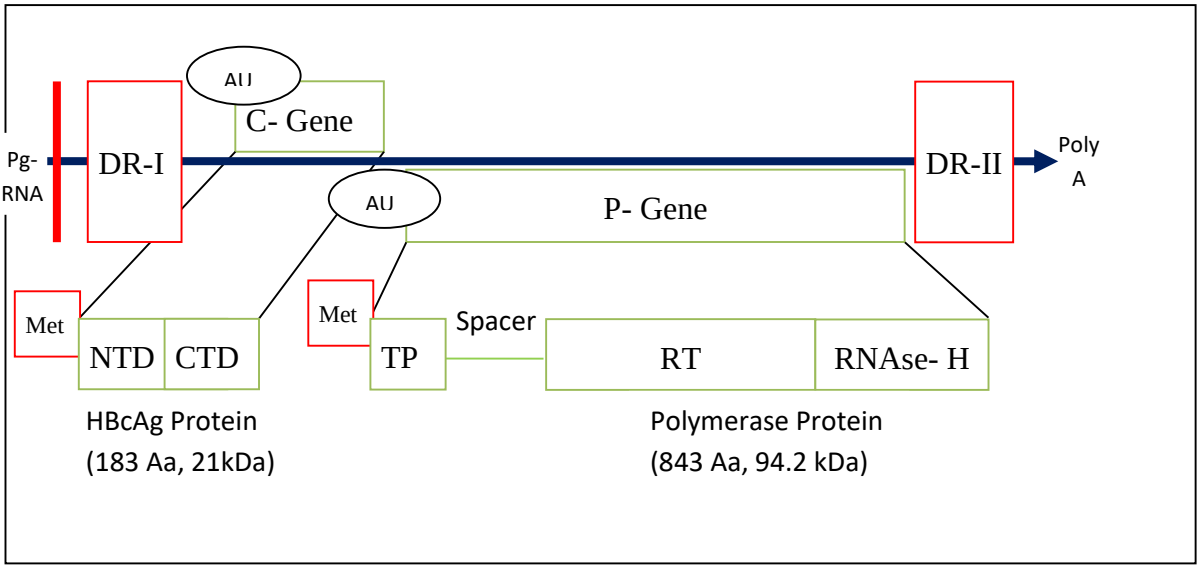

Figure no 2: pg-RNA, 3.4 kb. pg-RNA is organized in a bi-cistronic arrangement where the first AUG is encoding for core, and downstream, in a different reading frame, the AUG codes for the P protein. Core and P proteins are shown with their main domain organizations.

Core gene: Core gene encodes a protein (HBcAg) having a total number of 183 amino acids and the molecular weight of the core protein is about 21 kDa**.** The location of the core gene is near the 5′ end of pg-RNA and it contains its own start codon (AUG)**.** There is two structural and functional parts of this core protein, namely NTD (N-terminal domain) and CTD (C-terminal domain) (7).

- **NTD (N-terminal domain):** The N-terminal domain (NTD) of the core protein comprises 140 amino acids (residues 1–140). Its primary function is to mediate the self-assembly of HBcAg molecules into icosahedral nucleocapsids, thereby providing the structural framework of the virus—a process referred to as *capsid assembly*. This domain contains specific interface regions that enable core protein dimerization, which represents the initial step in capsid formation. During the assembly process, the NTD interacts with the viral polymerase (P protein) and pregenomic RNA (pgRNA) to initiate reverse transcription. The NTD, being exposed on the outer surface of the capsid, also constitutes the major immunogenic region of the core protein, recognized by host antibodies during infection. (figure no 2) (7).
- **CTD (C-terminal domain)**: The C-terminal domain (CTD) of the core protein consists of 43 amino acids (residues 141–183) and is rich in arginine residues. This domain plays a crucial role in viral replication by binding tightly to the pregenomic RNA (pgRNA) through its arginine-rich motifs, thereby functioning as an RNA chaperone that facilitates pgRNA encapsidation within the capsid. The CTD also contains nuclear localization signals (NLSs), which

enable the bidirectional transport of the capsid between the cytoplasm and the nucleus. Moreover, it regulates reverse transcription by ensuring the proper alignment of pgRNA and the polymerase (P) protein inside the nucleocapsid, a key step for viral DNA synthesis (Figure no. 2). In addition, the CTD possesses multiple phosphorylation sites; its phosphorylation status modulates RNA encapsidation, capsid maturation, and nuclear trafficking (8).

- **Direct Repeat (DR1 and DR2):** The Direct Repeat sequences (DR1 and DR2) within the Hepatitis B virus (HBV) genome are short, highly conserved regions that are essential for viral replication. They play a pivotal role in the reverse transcription of pregenomic RNA (pgRNA) into viral DNA, as well as in the processes of template switching, genome circularization, and packaging of the viral DNA within the nucleocapsid (Figure no. 2). Specifically, DR1 is positioned near the 5′ end of the pgRNA, immediately downstream of the ε (epsilon) stem-loop structure, while DR2 is located close to the 3′ end of the pgRNA (9).

P Gene (Polymerase protein): The P gene of HBV encodes the viral polymerase, a multifunctional protein composed of 843 amino acids with an approximate molecular mass of 94.2 kDa. Its translation begins at a downstream AUG codon and continues toward the 3′ end of the pregenomic RNA (pgRNA). The polymerase protein comprises four distinct structural and functional domains: the terminal protein (TP), spacer region, reverse transcriptase (RT), and RNase H domains (Figure no. 2) (9).

- **TP (Terminal Protein):** The terminal protein (TP) domain of the HBV polymerase consists of 180 amino acids (residues 1–180). It plays a critical role in initiating reverse transcription by serving as a protein primer for DNA synthesis. During this process, the TP domain becomes covalently attached to the 5′ end of the newly synthesized minus-strand DNA. A specific tyrosine residue (Y63 in HBV) within this domain acts as the primer for reverse transcription. DNA synthesis begins with the TP copying a short stretch of nucleotides using the ε (epsilon) stem-loop on the pregenomic RNA (pgRNA) as a template, and the TP remains covalently linked to the DNA throughout the replication cycle (10).
- **Spacer:** This domain contains the total number of 160 amino acids (181-340 AA). Location of this domain is between the terminal protein and reverse transcriptase protein so it acts like a flexible linker allowing the flexibility (11).
- **Reverse Transcriptase:** The reverse transcriptase (RT) domain of the HBV polymerase comprises 348 amino acids (residues 341–688) and is responsible for the core DNA polymerase activity. It utilizes the TP-linked primer to elongate the minus-strand DNA and subsequently initiates synthesis of the plus-strand DNA. This domain contains the highly conserved YMDD motif, which forms the catalytic site essential for polymerase function. Clinically, the RT domain is significant because it serves as the primary target for antiviral agents such as tenofovir and lamivudine (12).
- **RNase H:** The RNase H domain of the HBV polymerase consists of 155 amino acids (residues 689–843) and is responsible for degrading the pregenomic RNA (pgRNA) template during reverse transcription. As the minus-strand DNA is synthesized, RNase H cleaves the RNA strand within the RNA-DNA hybrid, leaving short RNA fragments. One of these fragments serves as the primer for plus-strand DNA synthesis. In the absence of RNase H activity, the RNA would remain bound to the DNA, preventing initiation of plus-strand DNA synthesis (13).

Pre Core-RNA (preC-RNA, 3.5 kb): The preC-RNA of HBV is a 3.5 kilobase transcript derived from the viral DNA, encompassing both the pre-core (E) and core regions. It contains two initiation codons (AUGs): the first directs translation of the pre-core region, while the second corresponds to the core gene, which encodes HBcAg. Unlike the pregenomic RNA (pgRNA), translation of preC-RNA begins at the upstream pre-core AUG rather than at the core AUG (Figure no. 3) (14).

Structural detail about preC-RNA, 3.5 kb:

- **Pre-core gene (E) and Core gene:** The pre-core (E) gene of HBV encodes a 29-amino-acid signal peptide, while the core gene (identical to the HBcAg coding sequence in the pregenomic RNA) contributes 183 amino acids, totaling 212 amino acids collectively. During translation, the combined product of the pre-core and core genes undergoes post-translational processing,

including cleavage in the endoplasmic reticulum and Golgi apparatus, followed by protein maturation and folding. This processing reduces the length to a 159-amino-acid protein, known as hepatitis B e antigen (HBeAg), with an approximate molecular weight of 18 kDa (15). HBeAg is secreted into the bloodstream, where its presence serves as a marker of active HBV infection (16).

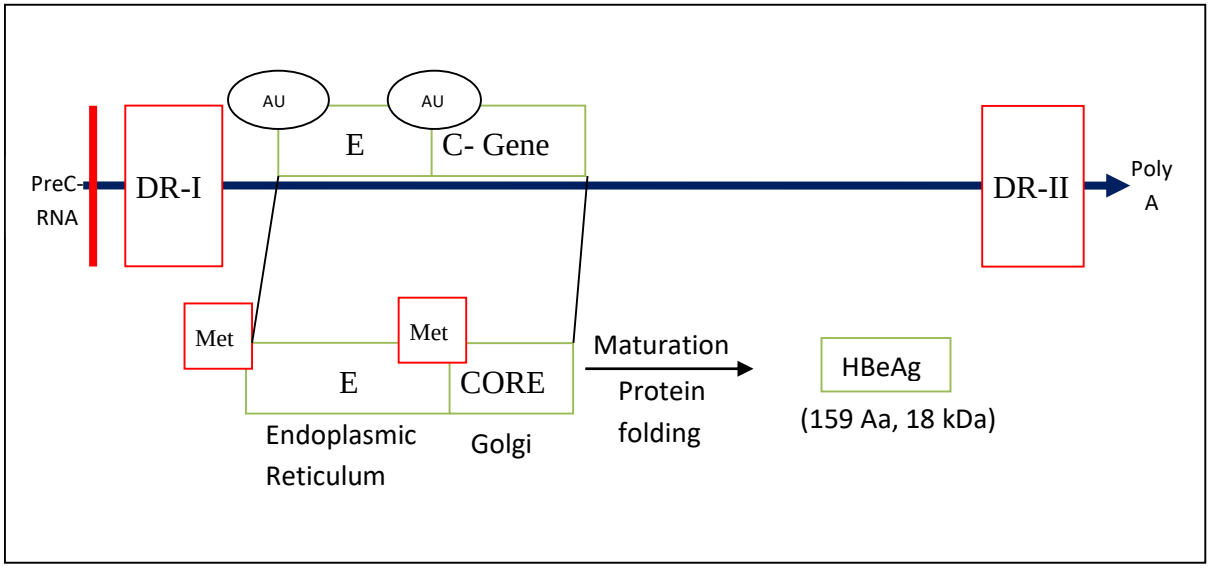

Figure no 3: preC-RNA, 3.5 kb. preC protein starts translation upstream to the core's AUG, but in the same reading frame. Sites for cleavage events (ER and Golgi apparatus) on the immature protein are shown. As a result, HBeAg is a secreted protein.

PreS1-RNA, 2.4 kb: The PreS1-RNA of HBV is a 2.4 kilobase transcript transcribed from the viral DNA and comprises three domains: Pre-S1 (108–119 amino acids), Pre-S2 (55 amino acids), and S (226 amino acids). This RNA encodes the large hepatitis B surface antigen (LHBs or Large HBsAg), a protein of 400 amino acids with an approximate molecular weight of 43.7 kDa. The LHBs protein is organized into three functional subdomains. The Pre-S1 domain binds to the NTCP receptor on hepatocytes, a critical step for viral entry into the liver. The Pre-S2 domain facilitates capsid assembly and mediates the secretion of large and middle surface proteins from the endoplasmic reticulum to the Golgi apparatus, aided by ER retention signals and hydrophobic regions. The S domain forms the structural framework of the viral envelope and contains the α-determinant, which is the primary target for neutralizing antibodies (Figure no. 4) (17).

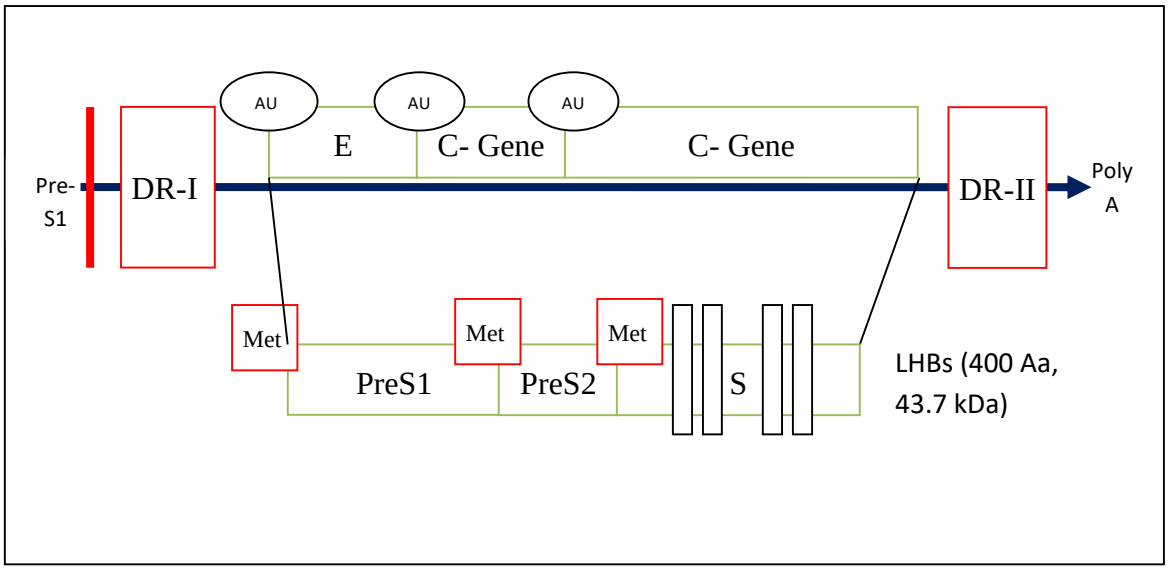

Figure no 4: preS1-RNA, 2.4 kb, preS1-RNA is predicted to encode the LHBs, although other AUGs are present in the same reading frame. LHBs is shown with its domains preS1, preS2, and S.

S-RNA, 2.1 kb: The S-RNA of HBV, a 2.1 kilobase transcript, is one of the shorter mRNAs produced from the viral genome. It includes two key regions: the Pre-S2 domain (55 amino acids) and the S domain (226 amino acids). The RNA contains two initiation codons (AUGs)—the first located in the Pre-S2 region and the second within the S region. Depending on which AUG is used to start translation, S-RNA can give rise to two distinct surface antigen isoforms. Thus, S-RNA contains two translation initiation sites that determine the specific protein product (Figure no. 5).

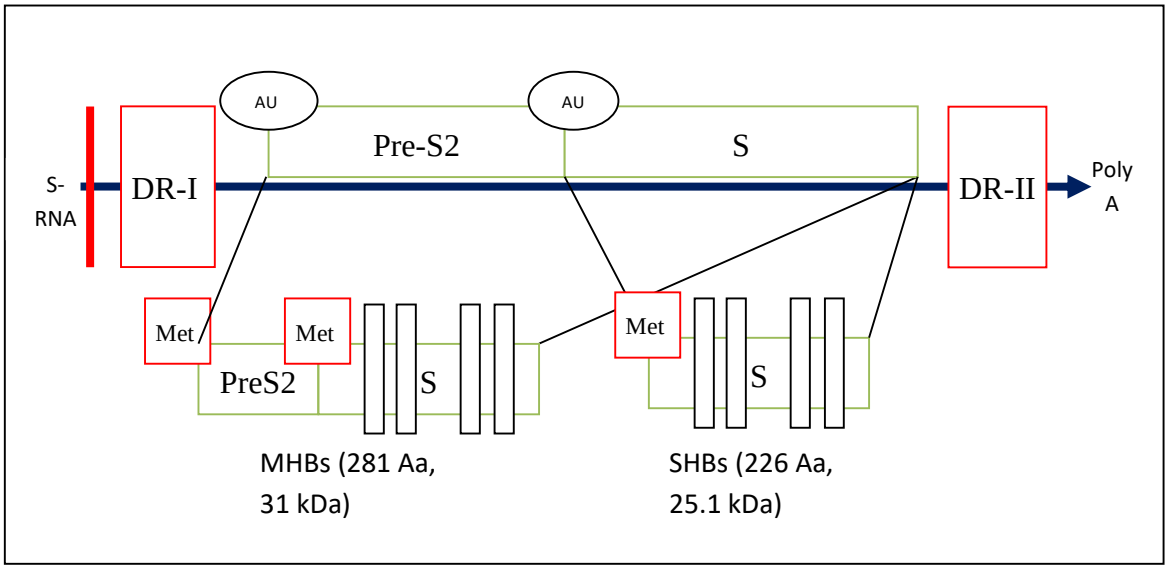

Figure no 5: S-RNA, 2.1 kb. S-RNA is predicted to encode both preS2 and S domains where MHBs and SHBs are produced.

Translation of MHBs (Middle HBsAg): Translation of S-RNA can initiate at the first AUG located in the Pre-S2 region, which results in the inclusion of both the Pre-S2 and S domains. This produces the middle hepatitis B surface antigen (MHBs), a protein composed of 281 amino acids with an approximate molecular weight of 31 kDa. Functionally, MHBs

plays a key role in the HBV life cycle by facilitating the secretion of viral and subviral particles and contributing to immune evasion, thereby helping the virus persist in the host. MHBs is incorporated into both Dane particles and subviral particles (SVPs) (Figure no. 5) (18).

Translation of SHBs (Small HBsAg): Translation of S-RNA can also begin at the second AUG located within the S region, producing the small hepatitis B surface antigen (SHBs). This protein consists solely of the S domain, containing 226 amino acids with an approximate molecular weight of 25.1 kDa. SHBs constitutes the main structural component of the viral envelope and is secreted in large quantities as non-infectious subviral particles (SVPs) (19).

Viral micro RNAs: MicroRNAs (miRNAs) are small, non-coding RNA molecules approximately 20–24 nucleotides in length that regulate gene expression by interacting with messenger RNAs (mRNAs), either inhibiting their translation or promoting their degradation. miRNAs are single-stranded RNAs derived from longer precursor hairpin structures. While the majority of miRNAs originate from the host genome, some are encoded by viruses (viral miRNAs). Both host and viral miRNAs exert post-transcriptional control over gene expression, influencing critical cellular processes such as the cell cycle, apoptosis, differentiation, and immune responses. Dysregulation of miRNAs has been strongly associated with cancer, viral infections, metabolic disorders, and neurological diseases.

Formation of HBV Viral miRNAs:

Transcription of HBV Viral miRNA genes (in Nucleus): MicroRNAs (miRNAs) constitute a class of small RNAs that play a pivotal role in gene silencing within complex regulatory networks (20). In humans, the majority of miRNAs are located within introns of protein-coding genes or within long noncoding transcripts. Depending on the alternative splicing patterns of the pre-mRNA, miRNAs may also be found in exons or introns. Additionally, some miRNAs originate from intergenic regions, situated between two protein-coding genes on the chromosome (21).

HBV-encoded viral miRNAs are located within introns of viral genes, indicating that their transcription is carried out by RNA polymerase II in the nucleus, resulting in transcripts that possess 5′ caps and 3′ poly(A) tails (22). Following transcription, a primary miRNA (pri-miRNA) is produced, typically 100–150 nucleotides long, with 1–4 nucleotide 3′ overhangs, 25–30 base pair stems, and small loops. The pri-miRNA adopts a hairpin structure containing specific signals for cleavage by double-stranded RNA-specific nucleases. In mammals, the Microprocessor complex, composed of Drosha and DGCR8, recognizes and processes this hairpin. Drosha cleaves the pri-miRNA in the nucleus, releasing a hairpin-shaped precursor known as pre-miRNA (23).

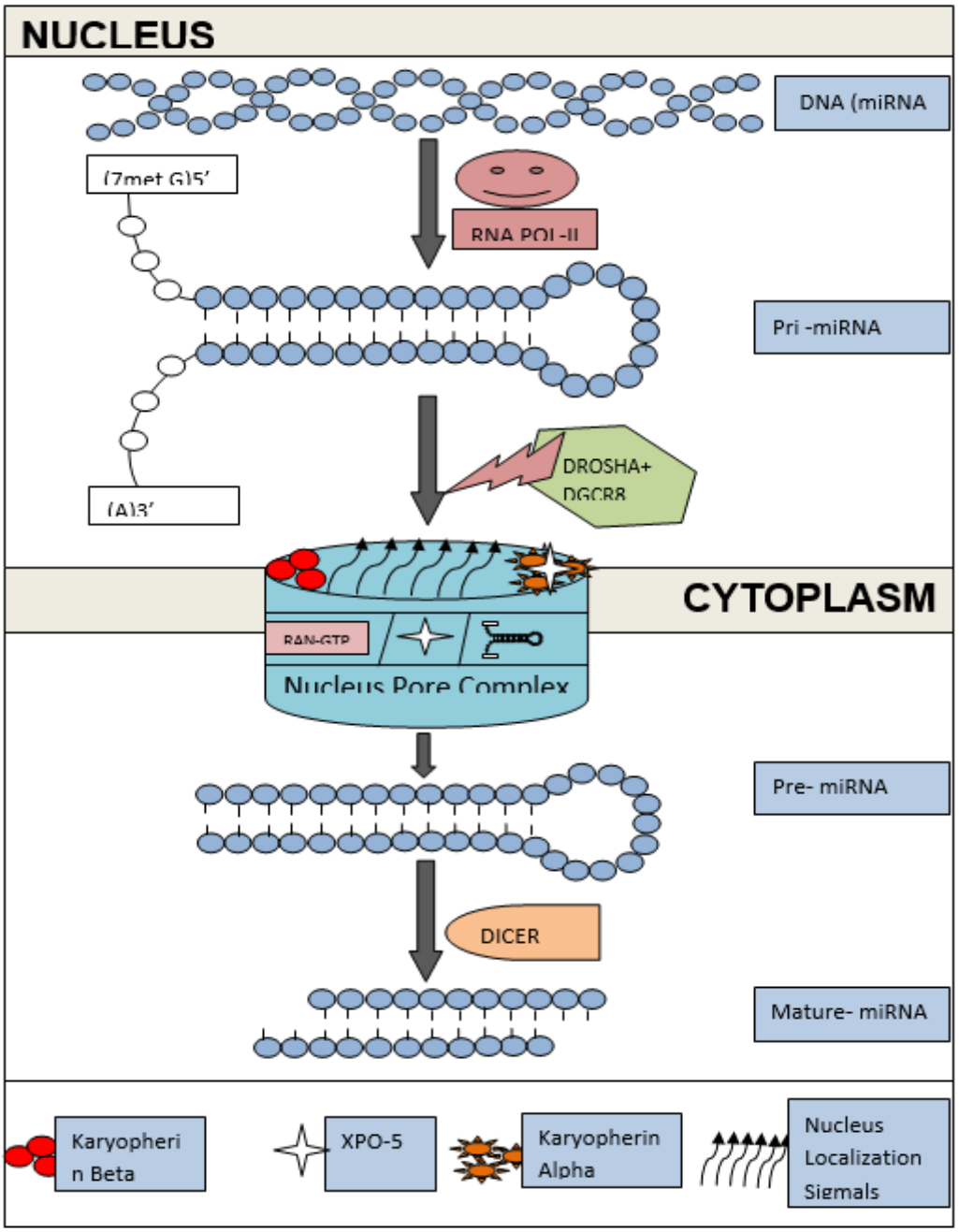

Figure no 6: Complete biogenesis of the Mature- miRNA from Pri-miRNA and Pre-miRNA and the entry of the Pri-miRNA from nucleus to cytoplasm.

XPO5 (Exportin-5), a member of the karyopherin-β family of nuclear transport receptors, serves as the primary mediator for the nuclear export of pre-miRNAs. It specifically recognizes the two-nucleotide 3′ overhang generated by Drosha cleavage at the base of pre-miRNA hairpins. The transport of pre-miRNA into the cytoplasm requires RanGTP, a GTP-binding protein that provides both energy and directionality for nuclear export (24). Initially, XPO5 binds the hairpin-shaped pre-miRNA and forms a ternary complex with RanGTP, which stabilizes the pre-miRNA and protects it from nuclease degradation (25). This XPO5–RanGTP–pre-miRNA complex is transported through the nuclear pore complex (NPC) from the nucleoplasm to the cytoplasm. Upon entry into the cytoplasm, RanGTP is hydrolyzed to RanGDP and inorganic phosphate (Pi) by RanGAP, inducing a conformational change in XPO5 that releases the pre-miRNA (26). The liberated pre-miRNA is then

recognized by Dicer, which cleaves it into a ~22 nucleotide double-stranded miRNA duplex (27).

Dicer, a member of the RNase III nuclease family, plays a key role in RNA interference. Once the pre-miRNA reaches the cytoplasm, Dicer cleaves it approximately 19 base pairs away from the Drosha cleavage site. This processing generates a double-stranded RNA duplex with 1–4 nucleotide 3′ overhangs at both ends. Of the two strands, only one is selected as the mature miRNA. In some cases, the mature miRNA originates from the leading strand of the pri-miRNA, while in others it derives from the lagging strand (28).

How miRNA controls the translations of targeted mRNA's:

The mature miRNA duplex is ultimately incorporated into the RISC-loading complex, which includes Dicer, Transactivation Response RNA-Binding Protein (TRBP), and Argonaute (Ago) proteins. Molecular chaperones assist in opening the Ago protein to adopt the proper conformation required for small RNA loading (29). Within Ago, the PAZ domain (first pocket) binds the 3′ two-nucleotide overhang of one strand, while the adjacent MID domain recognizes and anchors the 5′ monophosphate of the strand destined to become the guide. Selection of the guide strand is influenced by thermodynamic asymmetry: the strand with a less stably paired 5′ end is preferentially loaded into the MID pocket, as it is easier to unwind and position correctly. Additionally, Ago exhibits a preference for certain 5′ nucleotides, typically uracil (U) or adenine (A), further biasing the choice of the guide strand (30).

Removal of Passenger Strand:

- **Slicer-dependent passenger cleavage:** If the duplex is highly complementary, AGO2 cleaves the passenger strand opposite nucleotides 10–11 (counting from the guide 5′ end) and the cleaved passenger fragments are then rapidly degraded by exonucleases (31).
- **Slicer-independent unwinding:** The duplex is unwound by thermal "breathing" assisted by Ago and possibly helicase activity or chaperones; the passenger is ejected intact and then degraded and mainly the TRBP/PACT and chaperones facilitate this process (32).

Once the passenger strand is removed, the guide strand's 5′ phosphate is secured in the MID domain of Argonaute, while the 3′ end remains anchored in the PAZ domain or is released during conformational adjustments. These structural changes expose the seed region, comprising nucleotides 2–8 from the guide strand's 5′ end, enabling the complex to recognize and bind target RNAs. The resulting mature Argonaute–guide assembly is known as the RNA-induced silencing complex (RISC) (33). The RNA-induced silencing complex (RISC) is the active effector of gene silencing, formed when Argonaute (Ago) associates with a single-stranded guide RNA, such as a miRNA or siRNA. RISC employs the guide RNA to locate complementary sequences within target mRNAs. Upon target recognition, RISC can either cleave and degrade the mRNA if the pairing is perfect or near-perfect, or inhibit its translation if the pairing is partial, which is typical for most miRNAs. After silencing one target, RISC can dissociate and engage additional targets using the same guide RNA (34).

Role of viral miRNA's (miRNA-1, miRNA-2 and miRNA-3):

HBV-miR-1: HBV-miR-1 is a 22-nucleotide viral microRNA encoded primarily within the HBx region of the hepatitis B virus genome. This miRNA is located overlapping or adjacent to the HBx open reading frame (ORF) and the Enhancer I/Core promoter region of HBV DNA. It is expressed in infected hepatocytes and modulates both viral and host gene expression, thereby facilitating HBV replication, persistence, and contributing to oncogenic processes. The biogenesis pathway of HBV-miR-1 has been outlined previously (Figure no. 6) (35).

HBV-miR-1 downregulates PTEN (Phosphatase and Tensin Homolog), a tumor suppressor gene located on human chromosome 10q23. PTEN encodes a protein that normally inhibits the PI3K/AKT signaling pathway. However, in the presence of HBV-miR-1, PTEN expression is suppressed, leading to the accumulation of PIP3 and hyperactivation of PI3K/AKT signaling (36). Activated AKT phosphorylates and inactivates pro-apoptotic proteins such as BAD, BAX, and FOXO1, preventing damaged or infected hepatocytes from undergoing apoptosis. These long-lived, impaired cells accumulate mutations and genomic instability,

thereby increasing the risk of tumor initiation. Moreover, PI3K/AKT signaling activates mTOR (mammalian target of rapamycin) and transcription factors including NF-κB and CREB, driving uncontrolled hepatocyte proliferation and contributing to hepatocellular carcinoma (HCC) progression. This pathway also induces VEGF expression, promotes the Warburg effect, and suppresses interferon-stimulated genes (ISGs), further enhancing viral survival and oncogenesis (37).

Suppression of PTEN and subsequent overactivation of the PI3K/AKT pathway leads to the activation of transcription factors such as HNF4α and CREB, which bind to HBV Enhancer I/Core promoter regions. This enhances HBV replication by increasing pgRNA transcription and promoting viral DNA synthesis through reverse transcription. Additionally, HBV-miR-1 stabilizes HBs mRNA, boosting surface antigen production and virion secretion. By inhibiting apoptosis, infected hepatocytes survive longer, facilitating persistent viral replication (38).

Table no 1: Role of HBV-miRNA-1 in disease progression.

Level	Effect of HBV-miR-1	Outcome
Viral replication	Enhances pgRNA, core, and HBs mRNA expression	↑ Viral load
Host immune response	Downregulates antiviral genes	Immune evasion, chronic infection
Cell signaling	Activates PI3K/AKT/mTOR pathway	Increased hepatocyte survival
Oncogenesis	Suppresses PTEN, activates AKT → proliferation	Promotes HCC development

[1]pgRNA: Pre-genomic RNA.
[2]HBs: Hepatitis B surface.
[3]mTOR: mechanistic Target of Rapamycin.

HBV-miR-2: Hepatitis B virus microRNA-2 (HBV-miR-2) is a 22-nucleotide viral miRNA encoded by the HBV genome. It is situated within the surface antigen (S) gene, the preS region, or adjacent to the polymerase coding region. HBV-miR-2 functions as a regulatory miRNA, modulating both the viral life cycle and host pathways related to immunity and tumor development (39).

The primary viral target of HBV-miR-2 is the HBV polymerase gene, which overlaps the preS/S region. By binding to this site, HBV-miR-2 reduces polymerase mRNA translation, leading to a modest decrease in viral replication. Its secondary target is the HBV pregenomic RNA within the core promoter region (40). Interaction with this site slightly suppresses pgRNA synthesis when viral load is high, functioning as a feedback regulatory mechanism. These dual targeting actions allow HBV-miR-2 to act as a self-regulatory miRNA, modulating viral replication to prevent excessive viral production, evade immune detection, and promote chronic infection (41).

HBV-miR-2 also targets multiple host genes to manipulate cellular processes. It represses TRIM35 (Tripartite Motif-containing protein 35), suppresses RBL2 (Retinoblastoma-like protein 2), inhibits IRF2BP2 (Interferon Regulatory Factor 2 Binding Protein 2), and downregulates the cyclin-dependent kinase inhibitors p21 and p27. These combined effects reduce apoptosis, enhance cell survival, and promote tumorigenesis. Additionally, HBV-miR-2 fosters uncontrolled hepatocyte proliferation, diminishes antiviral immune responses, and supports both viral replication and liver cell proliferation (42).

HBV-miR-3: Hepatitis B virus microRNA-3 (HBV-miR-3) is a viral miRNA consisting of 21–23 nucleotides, encoded within the HBx gene region of the HBV genome. Its expression is notably higher in chronic HBV infections and HBV-associated hepatocellular carcinoma (HCC) compared to acute infections (43).

The viral targets of HBV-miR-3 include several regulatory functions. First, it binds to HBx mRNA within the HBx gene region, providing negative feedback that slightly reduces HBx expression and prevents excessive activation of host stress or immune responses. Second, HBV-miR-3 suppresses HBsAg mRNA, thereby controlling the production of viral surface proteins during chronic HBV infection. Third, it modulates overall viral replication, allowing the virus to persist at a stable, non-lethal level within hepatocytes (44).

Table no 2: Role of HBV-miRNA-3 in disease progression.

Mechanism	Outcome
PTEN suppression → ↑ PI3K/AKT	Cell survival and resistance to apoptosis
SOCS3 suppression → ↑ STAT3	Increased inflammation, angiogenesis, and proliferation
E-cadherin suppression	Epithelial-to-mesenchymal transition (EMT), invasion, metastasis
HBx fine-tuning	Chronic oxidative stress and DNA damage leading to carcinogenesis

[1]SOCS3: Suppressor of Cytokine Signaling 3
[2] STAT3: Signal Transducer and Activator of Transcription 3
[3] PTEN: Phosphatase and Tensin Homolog
[4] PI3K/AKT: Phosphatidylinositol 3-Kinase/Protein Kinase B

HBV-miR-3 also targets multiple host genes to manipulate cellular pathways. It suppresses SOCS3 (Suppressor of Cytokine Signaling 3), leading to increased STAT3 activity, enhanced cell proliferation, and tumor growth. It inhibits PTEN, resulting in activation of the PI3K/AKT pathway, increased cell survival, and reduced apoptosis. HBV-miR-3 also modulates IL-6 receptor (IL-6R) signaling, altering immune responses to favor chronic infection, and downregulates E-cadherin (CDH1), promoting invasion and metastasis in hepatocellular carcinoma (HCC) (44).

REFRENCES

1. Liang TJ. Hepatitis B The virus and disease. Hepatology. 2009;49:13-21.
2. Miller RH, Kaneko S, Chung CT, Girones R, Purcell RH. Compact organization of the hepatitis B virus genome. Hepatology. 1989;9:322-7.
3. Buti M, Rodriguez-Frias F, Jardi R, Esteban R. Hepatitis B virus genome variability and disease progression: The impact of pre-core mutants and HBV genotypes. J Clin Virol. 2005;34:79-82.
4. Kramvis A. Genotypes and Genetic Variability of Hepatitis B Virus. Intervirology. 2014;57:141-50.
5. Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. Intervirology. 2003;46:329-38.
6. Diab A, Foca A, Zoulim F, Durantel D, Andrisani O. The diverse functions of the hepatitis B core/capsid protein (HBc) in the viral life cycle Implications for the development of HBc targeting antivirals. Antivir. Res. 2018;149:211-20.
7. Jones SA, Hu J. Hepatitis B virus reverse transcriptase: diverse functions as classical and emerging targets for antiviral intervention. Emerg Microbes Infect. 2013 Sep;2(9):1-9.
8. Grice SL. Human immunodeficiency virus reverse transcriptase 25 years of research, drug discovery, and promise. J Biol Chem. 2012;287:40850-7.
9. Zhao Q, Chang J, Rijnbrand R, Lam AM, Sofia MJ, Cuconati A. Pregenomic RNA Launch Hepatitis B Virus Replication System Facilitates the Mechanistic Study of Antiviral Agents and DrugResistant Variants on Covalently Closed Circular DNA Synthesis. J of Virology. 2022;96(24):1-22.
10. Clark DN, Hu J. Hepatitis B Virus Reverse Transcriptase- Target of Current Antiviral Therapy and Future Drug Development. Antiviral Res. 2022;123:132-7.
11. Pley C, Lourenco J, McNaughton AL, Matthews PC. Spacer Domain in Hepatitis B Virus Polymerase: Plugging a Hole or Performing a Role. J of virology. 2022;96(9):1-14.
12. Clark DN, Flanagan JM, Hua J. Mapping of Functional Subdomains in the Terminal Protein Domain of Hepatitis B Virus Polymerase. J of Virology. 2017;91(3):1-15.
13. Cheng H, Zhang HZ, Shen WA, Liu YF, Ma FC. Expression of RNase H of human hepatitis B virus polymerase in Escherichia coli. World J Gastroenterol. 2003;9(3):1-3.
14. Yaginuma K, Shirakata Y, Kobayashi M, Koike K. Hepatitis B virus (HBV) particles are produced in a cell culture system by transient expression of transfected HBV DNA. Proc Natl Acad Sci. 1987;84:2678-82.
15. Standring D, Ou J, Masiarz F, Rutter W. A signal peptide encoded within the precore

region of hepatitis B virus directs the secretion of a heterogeneous population of e antigens in Xenopus oocytes. Proc Natl Acad Sci. 1988;85:8405-9.
16. Milich D, Liang T. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. Hepatology. 2003;38:1075-86.
17. Raney A, Milich D, Easton A, McLachlan A. Differentiation-specific transcriptional regulation of the hepatitis B virus large surface antigen gene in human hepatoma cell lines. J Virol. 1990;64:2360-8.
18. Ou J, Rutter W. Regulation of secretion of the hepatitis B virus major surface antigen by the preS-1 protein. J Virol. 1987;61:782-6.
19. Zajakina A, Kozlovska T, Bruvere R, Aleksejeva J, Pumpens P, Garoff H. Translation of hepatitis B virus (HBV) surface proteins from the HBV pregenome and precore RNAs in Semliki Forest virus-driven expression. J Gen Virol. 2004;85:3343-51.
20. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. Genome Res. 2004;14:1902-10.
21. Saini HK, Enright AJ, Griffiths-Jones S. Annotation of mammalian primary microRNAs. BMC Genomics. 2008;9:564:1-19.
22. Cai X, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA. 2004;10:1957-66.
23. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates miRNA processing. Nature. 2003;425:415-19.
24. Fischer T, Rodriguez-Navarro S, Pereira G, Racz A, Schiebel E, Hurt E. Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. Nature Cell Biology. 2004;6(9):840-44.
25. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev. 2003;17:3011-16.
26. Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA. 2004;10(2):185-91.
27. Feng Y, Zhang X, Graves P, Zeng Y. A comprehensive analysis of precursor microRNA cleavage by human Dicer. Published by Cold Spring Harbor Laboratory Press. 2012;18:2083–92.
28. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science. 2004;303(5654):95-8.
29. Iwakawa HO, Tomari Y. Life of RISC: Formation, action, and degradation of RNA-induced silencing complex. Mol Cell. 2022;82(1):30-43.
30. Shi CY, Kingston ER, Kleaveland B, Lin DH, Stubna MW, Bartel DP. The ZSWIM8 ubiquitin ligase mediates target-directed microRNA degradation. Science. 2020 Dec 18;370:1-28.
31. Betancur JG, Yoda M, Tomari Y. miRNA-like duplexes as RNAi triggers with improved specificity. Front Genet. 2012;3:127:1-6.
32. Park JH, Shin C. Slicer-independent mechanism drives small-RNA strand separation during human RISC assembly. Nucleic Acids Res. 2015;43(19):9418-33.
33. Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. Nat Rev Mol Cell Biol. 2019;20(1):5-20.
34. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010;79:351-79.
35. Momeni M, Hassanshahi G, Arababadi MK, Kennedy D. Ectopic expression of micro-RNA-1, 21 and 125a in peripheral blood immune cells is associated with chronic HBV infection. Mol Biol Rep. 2014;41(7):4833-7.
36. Tan YL, Chen WN. MicroRNAs as therapeutic strategy for hepatitis B virus-associated hepatocellular carcinoma: current status and future prospects. World J Gastroenterol. 2014;20(20):5973-86.
37. Xie KL, Zhang YG, Liu J, Zeng Y, Wu H. MicroRNAs associated with HBV infection and HBV-related HCC. Theranostics. 2014;4(12):1176-92.
38. Georgescu MM. PTEN Tumor Suppressor Network in PI3K-Akt Pathway Control. Genes Cancer. 2010;1(12):1170-7.

39. Yang X, Li H, Sun H, Fan H, Hu Y, Liu M, Li X, Tang H. Hepatitis B Virus-Encoded MicroRNA Controls Viral Replication. J Virol. 2017;91(10):1919-16.
40. Sartorius K, Makarova J, Sartorius B, An P, Winkler C, Chuturgoon A, et al. The Regulatory Role of MicroRNA in Hepatitis-B Virus-Associated Hepatocellular Carcinoma (HBV-HCC) Pathogenesis. Cells. 2019;8(12):1-35.
41. Thirion M, Ochiya T. Roles of microRNAs in the hepatitis B virus infection and related diseases. Viruses. 2013;5(11):2690-703.
42. Zulian V, Fiscon G, Paci P, Garbuglia AR. Hepatitis B Virus and microRNAs: A Bioinformatics Approach. Int J Mol Sci. 2023;24:1-28.
43. Shruti Chowdhari, Auroni Deep, Belal Ahmad, Jasmine Samal, Ekta Gupta, Perumal Vivekanandan, HBV-miR-3 induces hepatic cholesterol accumulation by targeting ABCA1: Evidence for potential benefits of statin usage, Journal of Lipid Research. 2025;66:8:1-14.
44. Gu S, Jin L, Zhang F, Sarnow P, Kay MA. Biological basis for restriction of microRNA targets to the 3' untranslated region in mammalian mRNAs. Nat Struct Mol Biol. 2009;16(2):144-50.