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HBV Pre-S Mutants and Hepatocellular Carcinoma: New Molecular Pathways, Clinical Implications, and Therapeutic Horizons (2020–2025)

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ABSTRACT

Hepatitis B virus (HBV) pre-S mutants, particularly deletions in the pre-S1 and pre-S2 regions of the viral envelope gene, have emerged as critical molecular drivers of hepatocellular carcinoma (HCC). These mutants contribute to liver carcinogenesis by inducing endoplasmic reticulum (ER) stress, oxidative DNA damage, and activating multiple oncogenic signaling pathways including PI3K/Akt/mTOR, NF-κB, and COX-2. Epidemiological data from 2020–2025 highlight their high prevalence in East Asian populations, especially in HBV genotypes B and C, with strong associations to tumor development, recurrence, and poor prognosis. Advancements in next-generation sequencing and liquid biopsy technologies have enabled the non-invasive detection of pre-S mutants, enhancing their potential as biomarkers for early diagnosis, risk stratification, and therapeutic monitoring. Preclinical and clinical research has also identified novel therapeutic strategies targeting mutant-induced stress pathways, immune evasion mechanisms, and metabolic reprogramming. The integration of pre-S mutant profiling into precision oncology approaches is anticipated to transform HCC management, particularly in HBV-endemic regions.

Keywords

Hepatitis B Virus (HBV), Pre-S Mutants, Hepatocellular Carcinoma (HCC), Oncogenesis, ER Stress, Oxidative DNA Damage, HBV Genotypes, Biomarkers, Next-Generation Sequencing (NGS), Liquid Biopsy, Immune Evasion, Precision Oncology, Tumour Recurrence, Pre-S1 Deletion, Pre-S2 Deletion.

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1. Introduction

1.1 Global Burden of Hepatocellular Carcinoma (HCC) and the Role of Hepatitis B Virus (HBV)

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and represents a major global health concern. According to GLOBOCAN 2020 estimates, HCC accounts for over 900,000 new cancer cases and approximately 830,000 deaths annually, making it the third leading cause of cancer-related deaths worldwide. The disease

disproportionately affects countries in East Asia and sub-Saharan Africa, regions where hepatitis B virus (HBV) infection remains endemic.[1]

Chronic HBV infection is the leading risk factor for HCC, contributing to more than 50% of HCC cases globally and up to 80% in high-prevalence areas. The virus exerts its oncogenic potential through both indirect and direct mechanisms. Indirectly, HBV induces chronic inflammation, hepatocyte regeneration, and fibrosis processes that eventually culminate in cirrhosis and malignant transformation. Direct mechanisms include integration of viral DNA into the host genome, leading to genetic instability, and expression of viral oncogenic proteins such as HBx and mutated surface antigens that interfere with tumor suppressor pathways.[2]

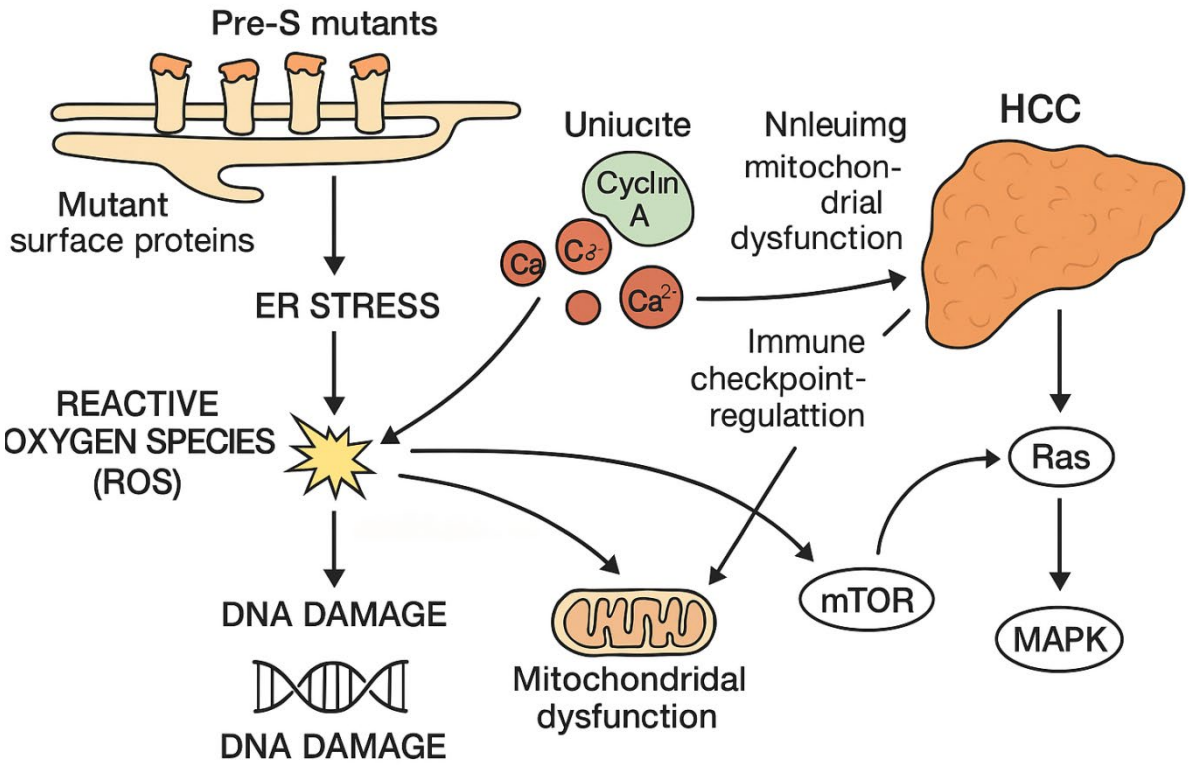

Figure 1: Global Distribution and Burden of Hepatocellular Carcinoma

1.2 Importance of Viral Mutations in Cancer Risk

The HBV genome is prone to mutations, especially under immune pressure or antiviral therapy. These adaptive mutations allow the virus to persist in the host, evade immune surveillance, and increase its oncogenic capacity. Among the most studied are mutations in the pre-core, basal core promoter (BCP), and the pre-S/S regions, each with distinct clinical implications.[3-5]

Mutations in the pre-S region, particularly deletions in pre-S1 and pre-S2 domains, have emerged as critical determinants of hepatocarcinogenesis. These mutants can accumulate in hepatocytes and lead to endoplasmic reticulum (ER) stress, oxidative DNA damage, chromosomal instability, and ultimately malignant transformation. Moreover, such mutations often arise during the immune-tolerant or inactive phases of HBV infection, making them silent yet lethal contributors to cancer risk.

Recent studies have shown that pre-S mutants are independently associated with increased risk of HCC development, recurrence after surgery, and poor clinical outcomes. Their presence in serum or liver tissue is now being investigated as a potential biomarker for early detection, prognosis, and therapeutic response.[6-7]

Table 1: Major HBV Mutations and Their Oncogenic Roles in Hepatocellular Carcinoma

Mutation Region	Common Mutations	Mechanism of Action	Association with HCC
Pre-core	G1896A	HBeAg-negative variant, immune escape	Moderate
BCP	A1762T/G1764A	Downregulates HBeAg, increases replication	High
Pre-S1	Large deletions	ER stress, DNA damage, immune escape	Very High
Pre-S2	Deletion/mutation	Induces ROS, activates oncogenic pathways	Very High
HBx	Truncation mutations	Interferes with p53, Wnt/β-catenin pathways	High

1.3 Focus on Pre-S Mutants as Emerging Drivers and Biomarkers

In the past decade (especially from 2020–2025), pre-S mutants have garnered growing attention for their dual role as oncogenic drivers and biomarkers. These deletions, typically spanning nucleotides within the pre-S1 or pre-S2 region, result in aberrant expression of surface antigens, accumulation within the ER, and subsequent cytotoxicity. This leads to activation of various cancer-related signaling pathways, including PI3K/Akt, mTOR, NF-κB, and MAPK.[8-9]

Beyond their mechanistic role in hepatocarcinogenesis, pre-S mutants can be detected through non-invasive methods, such as PCR-based assays from serum samples. This makes them

attractive targets not only for risk stratification but also for personalized therapeutic strategies, particularly in patients undergoing curative resection, transplantation, or antiviral therapy.

Ongoing translational research is focusing on targeting ER stress pathways, modulating host immune responses, and developing therapies that counteract the oncogenic potential of these mutants. As the molecular understanding deepens, pre-S mutants may soon occupy a central position in HCC screening programs and precision oncology protocols.[10-11]

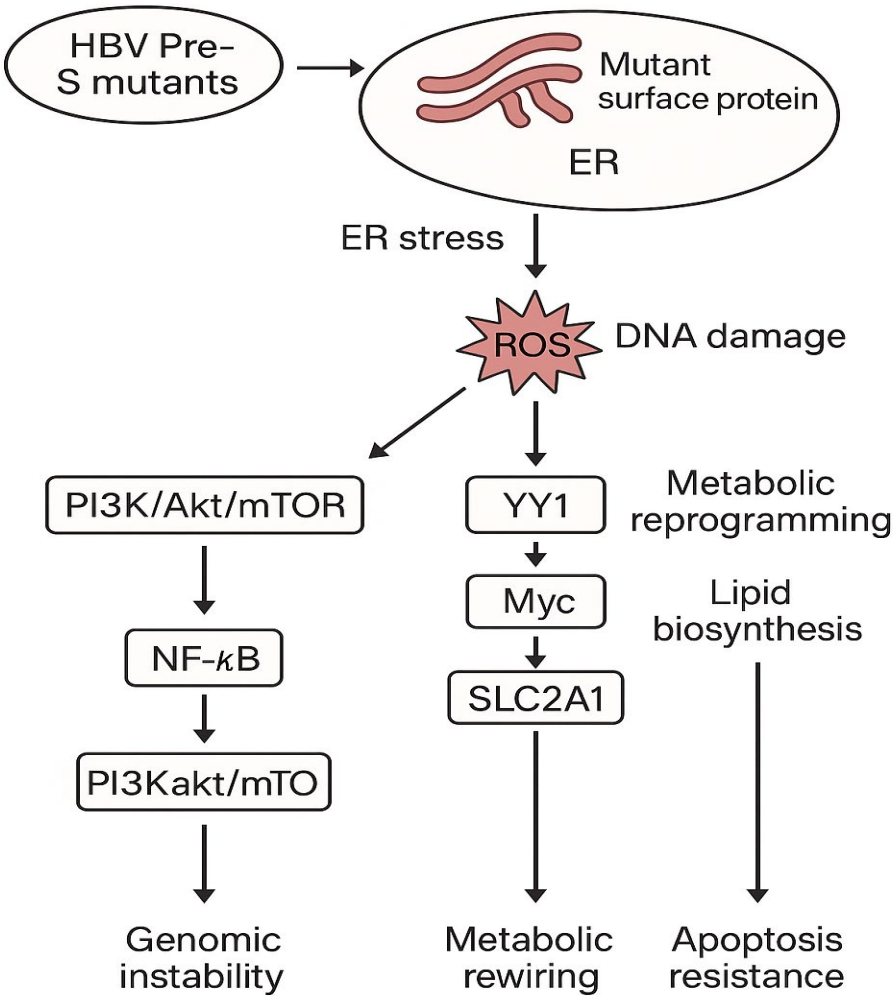

Figure 2 : Molecular Pathways Activated by HBV Pre-S Mutants in Hepatocarcinogenesis

2. HBV Pre-S Mutants: Structure and Evolution

2.1 HBV Genome and Surface Protein Gene Organization

Hepatitis B virus (HBV) is a small, partially double-stranded DNA virus belonging to the family Hepadnaviridae. Its 3.2 kb genome is organized into four overlapping open reading frames (ORFs): S (surface), C (core), P (polymerase), and X (transactivator)**.** Among these, the S ORF encodes the envelope proteins large (LHBs), middle (MHBs), and small (SHBs) surface antigens which are crucial for viral entry, replication, and immune recognition.

The surface gene consists of three in-frame start codons:

- **Pre-S1 domain**: Encodes part of the LHBs protein; involved in receptor binding (NTCP receptor)
- **Pre-S2 domain**: Shared by LHBs and MHBs; modulates immune responses
- **S domain**: Common to all three proteins; forms the SHBs antigen (HBsAg) [12]

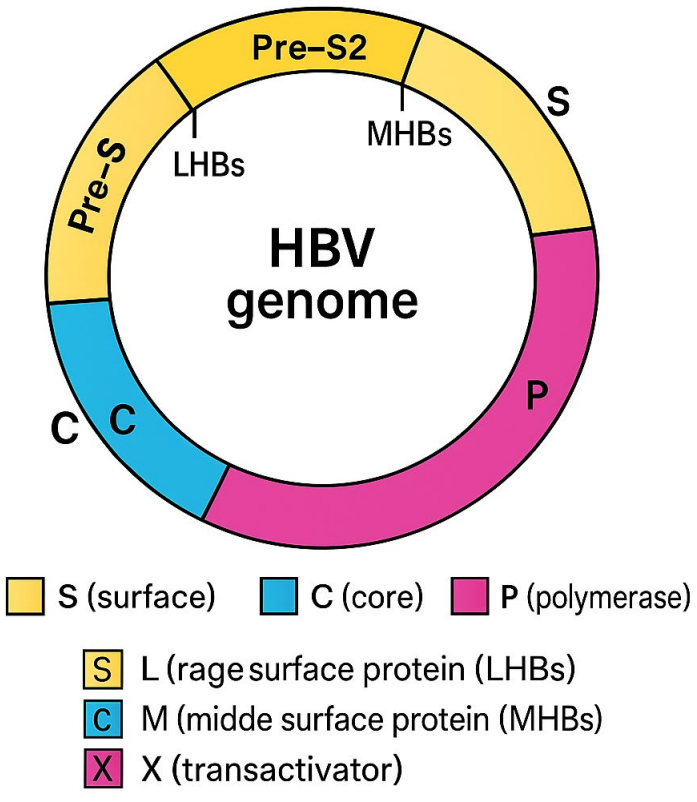

Figure 3 : Schematic Diagram of HBV Genome and Surface Protein Gene Organization

Mechanisms Generating Pre-S1 and Pre-S2 Deletions

Pre-S mutants arise primarily from deletion mutations in the pre-S1 or pre-S2 domains of the LHBs gene. These deletions are believed to be driven by:

- **Immune selection pressure** during chronic HBV infection
- **Error-prone reverse transcription** by HBV polymerase
- **Prolonged inflammation** and oxidative stress in hepatocytes

The deletions vary in size and location but typically involve:

- The N-terminal region of pre-S1, which disrupts receptor-binding and secretion
- The central to C-terminal region of pre-S2, affecting B-cell and T-cell epitopes

Such mutations lead to the intracellular accumulation of truncated LHBs proteins**,** particularly in the endoplasmic reticulum (ER), where they initiate ER stress, unfolded protein responses (UPR), oxidative damage, and ultimately oncogenic transformation.[13-15]

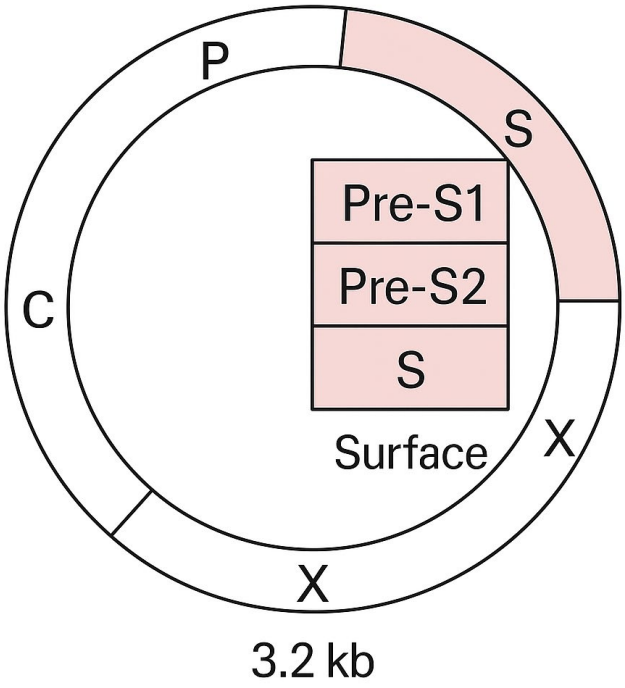

Figure 4 : Mechanism of Pre-S Mutant Formation and Their Cellular Consequences Genotype-Specific Patterns and Epidemiological Trends (2020–2025 Data)

HBV is classified into at least **10 genotypes (A–J)**, which differ by more than 8% in genomic sequence and show distinct geographic distributions. The frequency, location, and oncogenic potential of pre-S deletions vary according to genotype:

- **Genotype B** (common in East Asia): Associated with pre-S2 deletions, higher prevalence of ground-glass hepatocytes (GGHs), and early-onset HCC.
- **Genotype C** (East and Southeast Asia): Frequently shows pre-S1 deletions, later-onset but more aggressive HCC, and higher mutation rates.
- **Genotype D** (Mediterranean, Middle East, India): Moderate prevalence of pre-S deletions; associated with cirrhosis-related HCC.
- **Genotype A & E** (Africa, Europe): Lower frequency of pre-S deletions; unclear oncogenic significance.

Recent studies from 2020–2025 using next-generation sequencing (NGS) and longitudinal cohort analyses have strengthened the link between genotype-specific mutation profiles and clinical outcomes**,** such as recurrence-free survival, tumor size, and treatment response[15-20]

Table 2: Genotype-Specific Pre-S Mutant Profiles and Clinical Associations (2020–2025)

HBV Genotype	Common Pre-S Mutant	Mutation Region	Geographic Region	Clinical Significance
B	ΔPre-S2 (60–120 aa)	C-terminal	China, Taiwan	Early HCC, high recurrence
C	ΔPre-S1 (1–50 aa)	N-terminal	Japan, Korea, Vietnam	Late HCC, high tumor burden
D	ΔPre-S1 & Pre-S2	Mixed	India, Middle East	Moderate oncogenic risk
A	Rare	Sporadic	Europe, Sub-Saharan Africa	Limited association with HCC
E	Rare	Undefined	West Africa	Poorly studied

3. Clinical Relevance of Pre-S Mutants

3.1 Prevalence in Chronic HBV, Cirrhosis, and HCC (Recent Cohort Data)

Pre-S mutants particularly deletions in pre-S1 and pre-S2 regions have been increasingly documented in the continuum of HBV-related liver disease. A growing body of cohort-based research from 2020–2025 provides strong evidence for their progressive enrichment across the clinical spectrum:

- **Chronic HBV without cirrhosis**: 10–25% prevalence
- **HBV with compensated cirrhosis**: 30–40%
- **HBV-related HCC**: Up to 60–75% in some East Asian populations

This trend highlights a positive correlation between pre-S mutant prevalence and disease severity**.** Their presence is particularly high in HBV genotype B and C populations, notably in China, Taiwan, South Korea, and Vietnam. Several longitudinal cohort studies (e.g., China Liver Cancer Project, 2021–2024) have confirmed that patients harboring pre-S2 deletions are significantly more likely to progress from inactive carrier status to HCC within 5–10 years.[20-25]

Table 3 : Prevalence of Pre-S Mutants Across HBV Disease Stages (Data: 2020–2025 Cohorts)

Clinical Stage	Prevalence of Pre-S Mutants	Key Study/Region
Chronic HBV (no fibrosis)	10–25%	Taiwan (Liu et al., 2021)
HBV with cirrhosis	30–40%	Korea (Lee et al., 2022)
HBV-related HCC	60–75%	China (Zhang et al., 2023)
Post-HCC resection	>80% (recurrence risk)	Japan (Kawakami et al., 2024)

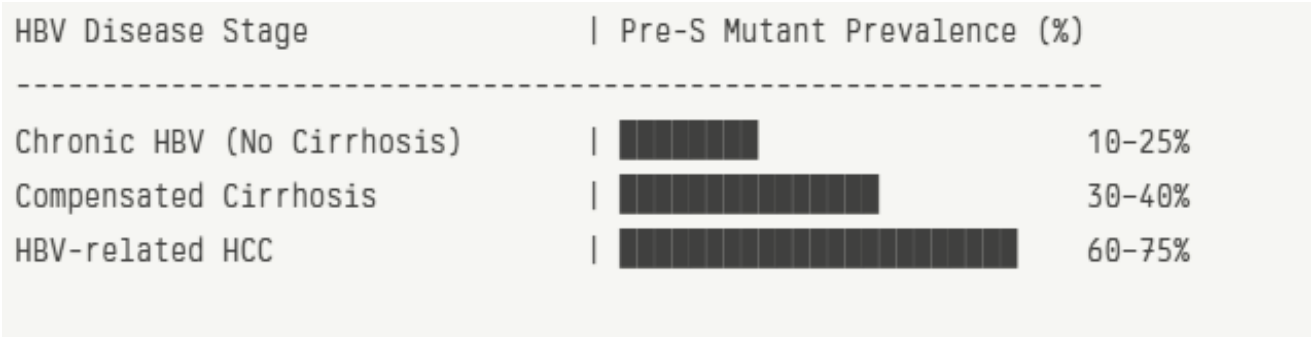

Figure 5 : Bar Graph Showing Increasing Pre-S Mutant Prevalence Across HBV Disease Spectrum

3.2 Prognostic Value for HCC Development and Recurrence

Pre-S mutants are not only associated with the presence of HCC but also hold strong prognostic value for predicting both initial tumor development and post-treatment recurrence**.**

- **Risk of HCC development**: Patients with pre-S2 deletions have a 2–3-fold higher risk of developing HCC compared to those without, even after adjusting for HBV DNA levels, age, and liver fibrosis.
- **Postoperative recurrence**: In resected HCC patients, detection of pre-S deletions (especially in intrahepatic tissue) correlates with a significantly reduced recurrence-free survival (RFS) and overall survival (OS)**.**

  Example: A 2023 meta-analysis (Zhou et al., Liver Int.) concluded that pre-S2 deletion positivity was an independent predictor of 1-year and 3-year recurrence.

- **Recurrence type**: Pre-S mutants are especially associated with early intrahepatic recurrence**,** suggesting their role in field cancerization and residual microtumor expansion.[25-30]

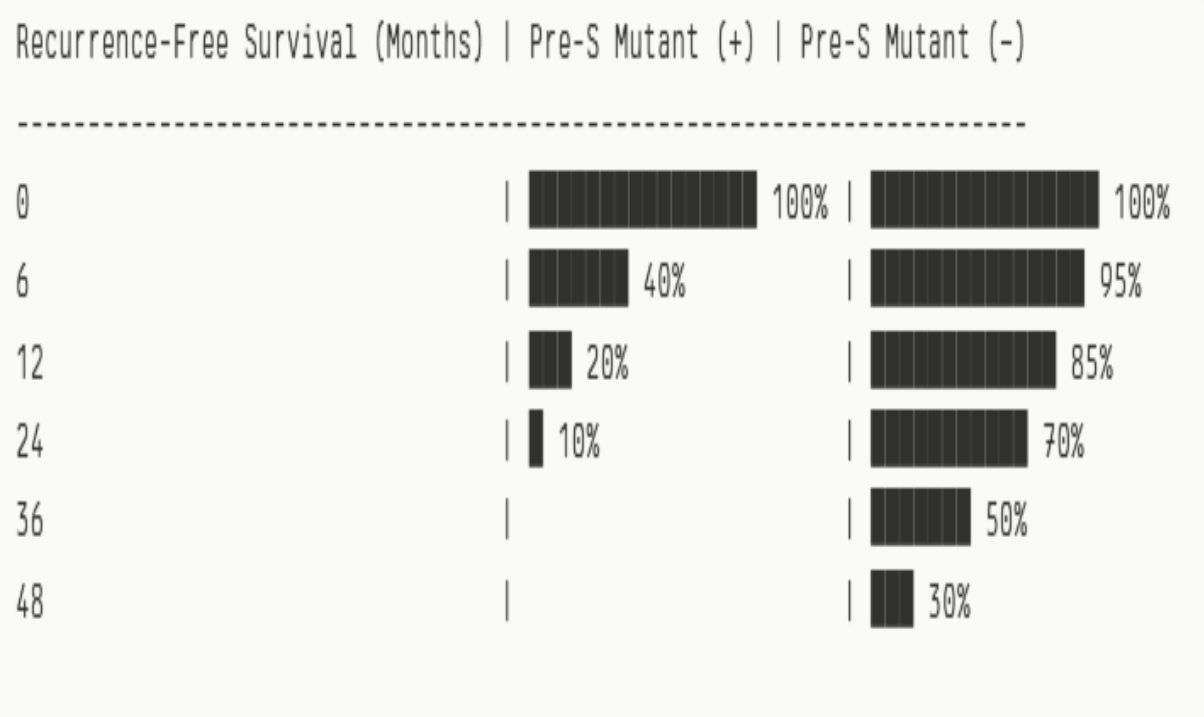

Figure 6: Kaplan-Meier Curves of RFS in Pre-S Mutant (+) vs. (–) HBV-HCC Patients

4. Advances in Detection: Next-Generation Sequencing and Plasma Biomarkers

4. 1. Next-Generation Sequencing (NGS):

The advent of **deep sequencing technologies** has revolutionized the detection of HBV variants, enabling:

- High-sensitivity detection of minor mutant clones
- Quantification of mutation burden
- Characterization of mutant heterogeneity across viral quasispecies

NGS panels designed specifically for HBV pre-S/S regions are now commercially available and have been implemented in clinical trials for HCC screening (e.g., HBVscan™, GenoLife Biotech).[30]

4. 2. Plasma-Based Biomarkers:

Beyond liver biopsy and sequencing, plasma-based detection methods have emerged as non-invasive, cost-effective alternatives**:**

- Circulating HBV DNA with pre-S deletions can be identified via digital PCR or NGS.
- Pre-S2 mutant protein fragments can be measured using ELISA or antibody-based sensors.
- Combination with AFP (alpha-fetoprotein) and des-γ-carboxy prothrombin (DCP) enhances early detection of HCC.[31]

Table 4: Diagnostic Tools for Pre-S Mutant Detection

Method	Sample Type	Sensitivity	Clinical Use
Sanger Sequencing	Liver tissue	Low	Research only
Digital PCR	Plasma	High	Clinical diagnostic labs
Targeted NGS Panels	Plasma	Very High	HCC risk stratification
ELISA for Mutant LHBs	Plasma	Moderate	Early screening in HBV patients

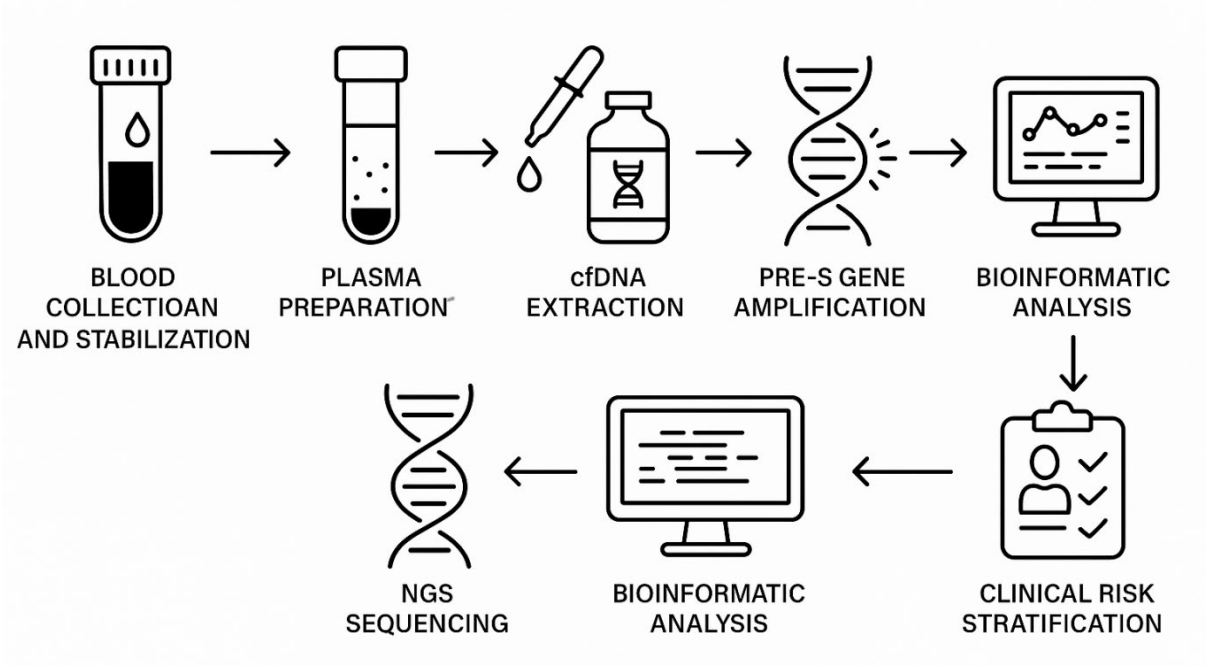

Figure 7: Workflow of Pre-S Mutant Detection Using Liquid Biopsy + NGS

4.3 . Molecular Mechanisms of Oncogenesis

The oncogenic potential of HBV pre-S mutants arises from their ability to interfere with intracellular homeostasis and reprogram multiple signaling cascades within hepatocytes. These effects go beyond traditional inflammation-driven models of carcinogenesis and instead involve precise molecular alterations**,** organelle dysfunction**,** and metabolic reprogramming**.** Here, we dissect the critical molecular mechanisms implicated in the pathogenesis of HBV-related HCC due to pre-S mutants.[32-35]

4.4 ER Stress and Unfolded Protein Response (UPR) Activation

The truncated pre-S1 and pre-S2 proteins resulting from deletion mutations in the HBV surface gene tend to misfold and accumulate in the endoplasmic reticulum (ER)**,** triggering a condition known as ER stress**.** This leads to activation of the Unfolded Protein Response (UPR)**,** which initially aims to restore proteostasis but eventually promotes cell death or oncogenesis if unresolved.[36-38]

Events:

- Accumulation of LHBs mutant proteins in ER lumen
- Activation of UPR sensors: IRE1, PERK, and ATF6
- Upregulation of chaperones (e.g., GRP78/BiP)

- Prolonged UPR → pro-inflammatory cytokine release, apoptosis evasion, and pro-survival gene activation

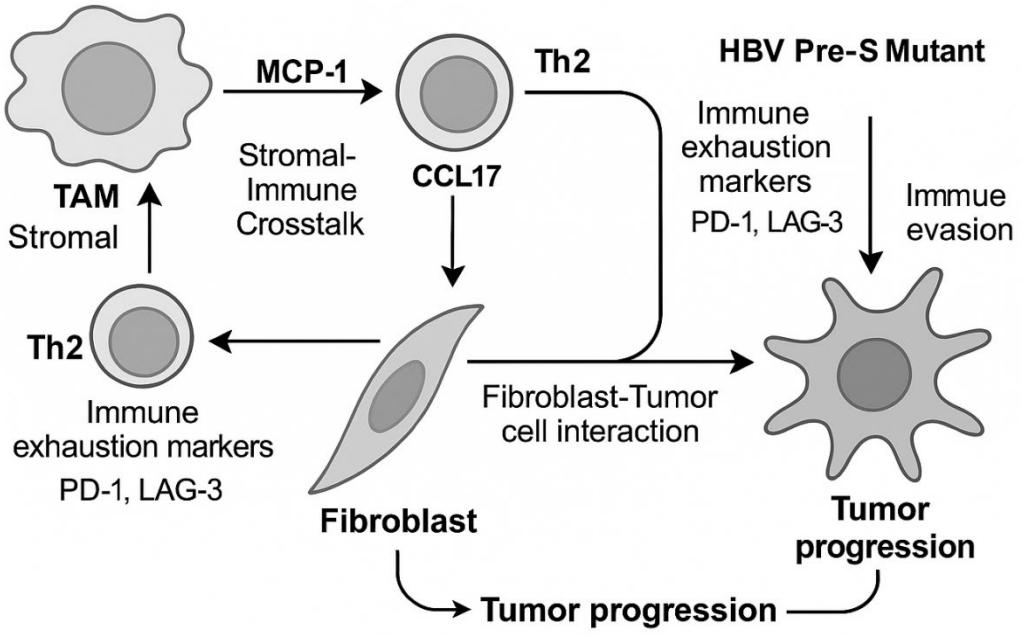

Figure 8 : Schematic of ER Stress and UPR Induced by HBV Pre-S Mutants

4.5 Oxidative Stress and Genomic Instability

The ER stress induced by pre-S mutant proteins contributes to mitochondrial dysfunction and excessive production of reactive oxygen species (ROS)**.** These ROS act as DNA-damaging agents**,** causing:

- Single- and double-strand breaks
- Point mutations and chromosomal aberrations
- Micronuclei formation and centrosome amplification

This persistent oxidative insult drives genomic instability, a hallmark of cancer. Additionally, ROS upregulate mutagenic and inflammatory pathways that further contribute to the oncogenic microenvironment [39-40]

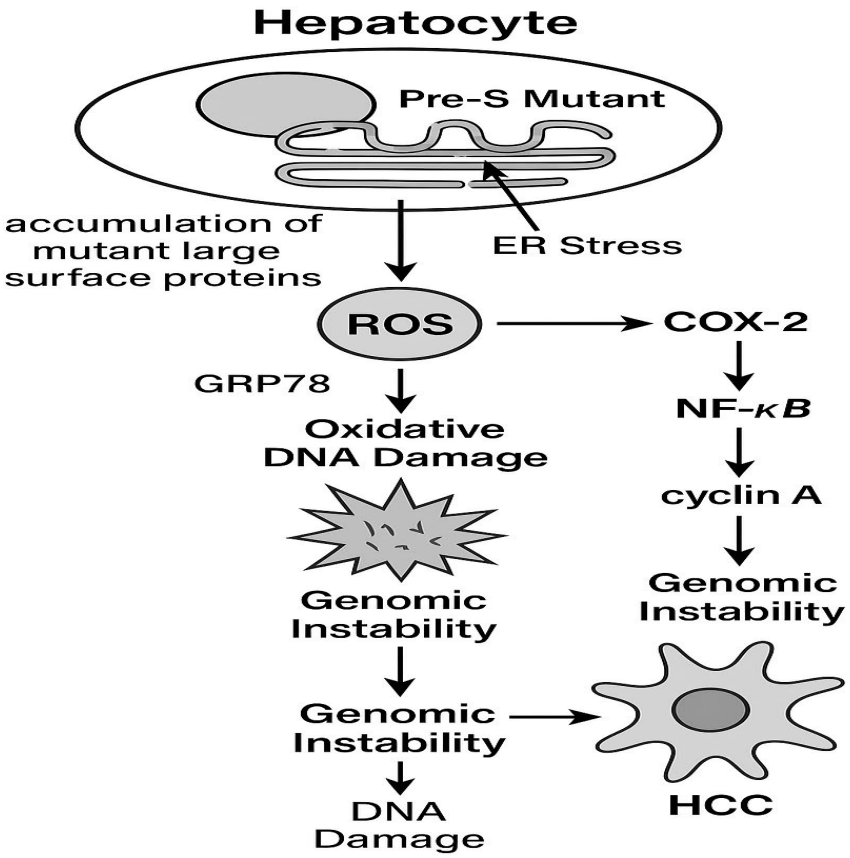

Figure 9 : Pathway of ROS Generation and Genomic Instability in Pre-S Mutant-Harboring Cells

4.6 Oncogenic Signaling Pathways

HBV pre-S mutants activate several oncogenic signaling cascades, many of which are also involved in inflammation, angiogenesis, metabolism, and immune evasion.

4.6.1 NF-κB / p38 MAPK / COX-2 Axis

- Activation of NF-κB and p38 MAPK leads to increased expression of COX-2, a pro-inflammatory enzyme associated with tumor promotion.
- This axis supports anchorage-independent growth, proliferation, and resistance to apoptosis.

4.6.2 VEGF-A / Akt / mTOR Pathway

- Upregulation of vascular endothelial growth factor A (VEGF-A) stimulates angiogenesis.

- VEGF-A activates PI3K-Akt-mTOR signaling → enhanced cell survival, proliferation, and metabolism.

4.6.3 YY1 / Myc / SLC2A1 Axis (Glycolysis)

- Pre-S mutants increase the expression of Yin Yang 1 (YY1), which promotes c-Myc-mediated upregulation of GLUT1 (SLC2A1), a key glucose transporter.
- This drives aerobic glycolysis (Warburg effect), favoring tumor cell energy demands.

4.6.4 SREBF1 / ACLY / FADS2 Axis (Lipid Metabolism)

- Pre-S mutants reprogram lipid biosynthesis via sterol regulatory element-binding protein 1 (SREBF1), leading to upregulation of ATP citrate lyase (ACLY) and fatty acid desaturase 2 (FADS2).
- These changes fuel membrane synthesis and oncogenic signaling. [41-50]

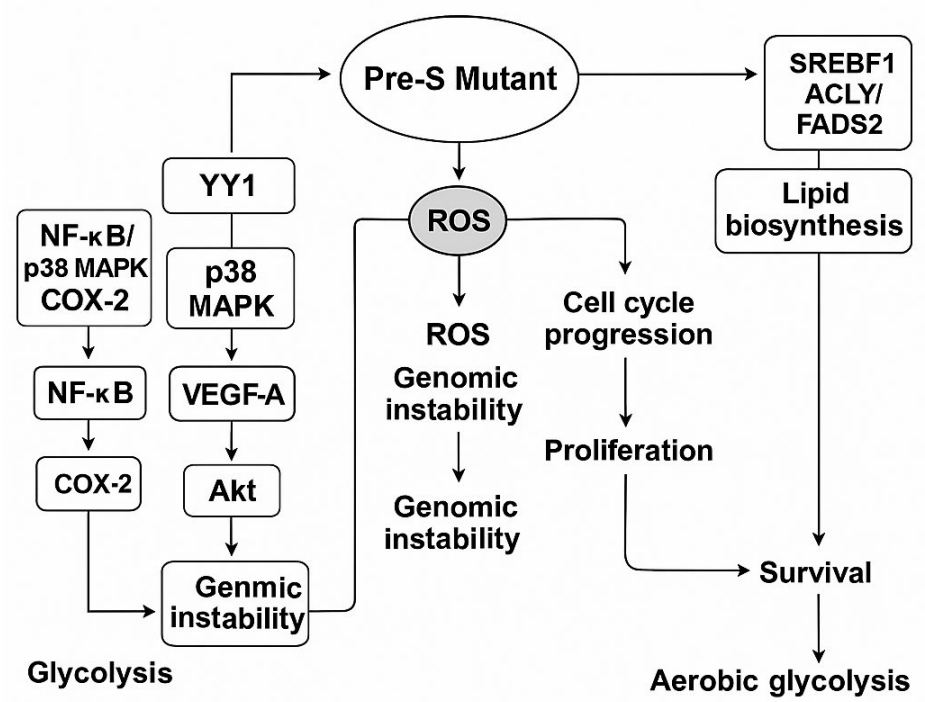

Figure 10 : Integrated Map of Oncogenic Signaling Pathways Activated by Pre-S Mutants

4.6.4 Unique Pathways in Pre-S2 Mutants

Pre-S2 mutants exhibit particularly aggressive phenotypes due to the activation of non-canonical and cell cycle-related oncogenic mechanisms**.**

4.6.5 Mitochondrial Dysfunction and Calcium Overload

- Accumulation of mutant proteins leads to disrupted calcium homeostasis between the ER and mitochondria.
- This results in mitochondrial Ca^{2+} overload, loss of membrane potential, and metabolic dysregulation.

4.6.6 Cyclin A Dysregulation and Centrosome Amplification

- Pre-S2 mutants promote overexpression of Cyclin A**,** resulting in unscheduled S-phase entry and centrosome amplification**,** contributing to aneuploidy.

4.6.7 JAB1 / p27 / Cdk2 / Rb Signaling

- Pre-S2 mutants activate JAB1 (Jun activation domain-binding protein 1**)**, which degrades p27**,** a cell cycle inhibitor.
- This enhances Cdk2/Rb phosphorylation, allowing unchecked cell cycle progression.

4.6.7 Impaired DNA Repair (NBS1 Transport Blockade)

- Pre-S2 mutants interfere with the nuclear import of NBS1, a critical component of the MRN DNA repair complex.
- This contributes to ineffective double-strand break repair and further genomic instability.

4.6.8 Bcl-2–Mediated Survival and Drug Resistance

- Pre-S2 mutants upregulate anti-apoptotic proteins like Bcl-2**,** enabling tumor cell survival under genotoxic stress and enhancing resistance to chemotherapy**.** [50-55]

Table 5 : Summary of Molecular Events Induced by Pre-S2 Mutants

Pathway/Event	Molecular Target/Effect	Functional Outcome	Reference
ER stress & UPR	IRE1, PERK, ATF6 activation	Pro-survival signals	56
ROS & genomic instability	DNA strand breaks, centrosome amplification	Genomic chaos	57
NF-κB / COX-2 axis	Inflammatory cytokines, COX-2 upregulation	Proliferation, immune escape	58

VEGF / mTOR signaling	Angiogenesis, cell growth	Tumor expansion	59
YY1 / Myc / SLC2A1 axis	GLUT1 expression → glycolysis	Metabolic reprogramming	60
SREBF1 / ACLY / FADS2 axis	Lipogenesis and membrane remodeling	Tumor cell growth	61
Cyclin A / Centrosome	Cell cycle dysregulation	Aneuploidy, proliferation	62
JAB1 / p27 / Cdk2 / Rb	Loss of checkpoint control	Unrestricted division	63
NBS1 transport disruption	Defective DNA damage response	Genomic instability	64
Bcl-2 pathway	Apoptosis inhibition	Drug resistance	65

4.6.9 Tumor Microenvironment and Immune Modulation

This section explores how HBV pre-S mutants reshape the tumor immune microenvironment (TME) to promote immune evasion, tumor growth, and recurrence focusing on checkpoint regulation, chemokines, and synergy with other viral oncogenic elements like truncated HBx.

5. Pre-S Mutants and Immune Checkpoint Regulation (PD-L1, Tregs)

One of the emerging hallmarks of cancer is the ability to evade immune destruction, and HBV pre-S mutants contribute to this via modulation of immune checkpoint pathways.[66-67]

Mechanisms:

- **PD-L1 Upregulation**: Pre-S mutant proteins (especially pre-S2 deletions) have been shown to induce PD-L1 expression on hepatocytes and antigen-presenting cells (APCs), creating an immunosuppressive barrier that inhibits cytotoxic $CD8^+$ T cell responses.
- **Treg Expansion**: Pre-S mutants indirectly promote the expansion and recruitment of regulatory T cells (Tregs) in the liver microenvironment by altering cytokine/chemokine secretion (e.g., increased IL-10**,** TGF-β**).**

These effects blunt the antiviral and anti-tumor immune response, allowing persistence of HBV-infected cells and the expansion of pre-malignant clones.[68-70]

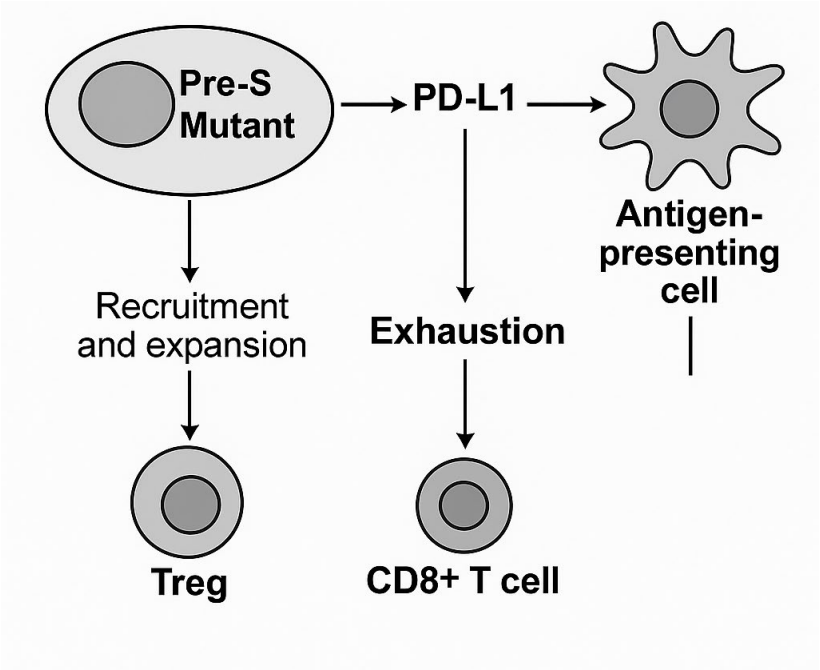

Figure 11 : Pre-S Mutant-Mediated Immune Checkpoint Regulation in the Liver Microenvironment

6. Chemokine Signatures (e.g., MCP-1) and Recurrence Risk

Recent transcriptomic and proteomic profiling (2020–2025) has identified distinct chemokine signatures in patients harboring pre-S mutants, which influence immune cell recruitment**,** inflammation**,** and tumor recurrence post-treatment.

Findings:

- Elevated monocyte chemoattractant protein-1 (MCP-1 / CCL2) levels are commonly observed in pre-S mutant-positive HCC patients.
    - MCP-1 attracts monocytes and tumor-associated macrophages (TAMs)**,** which promote tumor growth and angiogenesis.
    - High MCP-1 levels correlate with early recurrence and microvascular invasion after resection or RFA (radiofrequency ablation).
- Other chemokines linked include CCL17 (recruits Tregs) and CXCL12 (supports metastasis via CXCR4 interaction).[71-72]

Table 6: Chemokine Alterations Associated with Pre-S Mutants and HCC Outcomes

Chemokine	Cellular Source	Immune Effect	Clinical Impact	Reference
MCP-1	Hepatocytes, macrophages	Monocyte/TAM recruitment	↑ Recurrence, ↓ RFS	73
CCL17	Dendritic cells	Treg attraction	Immunosuppression	74
CXCL12	Tumor stromal cells	Invasion, angiogenesis	↑ Metastasis, ↓ OS	75

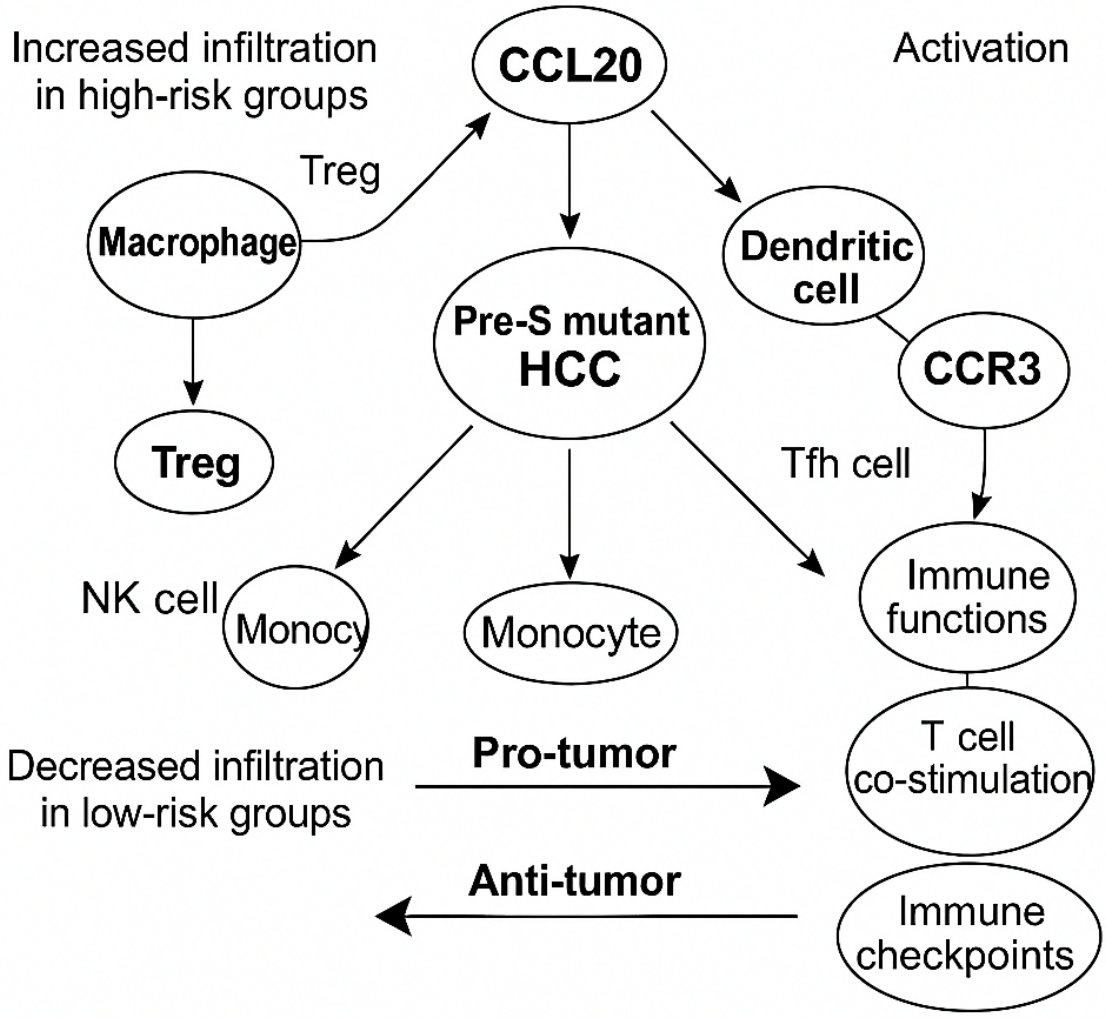

Figure 12 : Chemokine Network Alterations in the Pre-S Mutant–HCC Microenvironment

6.1 Interaction with Truncated HBx and Synergistic Oncogenesis

Pre-S mutants rarely act in isolation. In many HBV-HCC patients, co-expression of truncated HBx (ΔHBx) a mutated form of the viral X protein further accelerates hepatocarcinogenesis via synergistic interactions.[76]

Co-oncogenic Synergy:

- ΔHBx enhances transcriptional activation of oncogenes, DNA repair inhibition, and chromatin remodeling.
- When co-expressed with pre-S mutants:
  - ER stress and ROS levels are amplified, causing more severe DNA damage.
  - PD-L1 and VEGF expression is synergistically upregulated, promoting immune escape and angiogenesis.
  - Enhanced activation of β-catenin, STAT3, and TGF-β pathways.[77-79]

Experimental models (e.g., HBV-transgenic mice and 3D liver organoids) have shown that tumors co-expressing pre-S2 mutants and truncated HBx are larger, more vascularized, and more resistant to chemotherapy or immune checkpoint inhibitors.

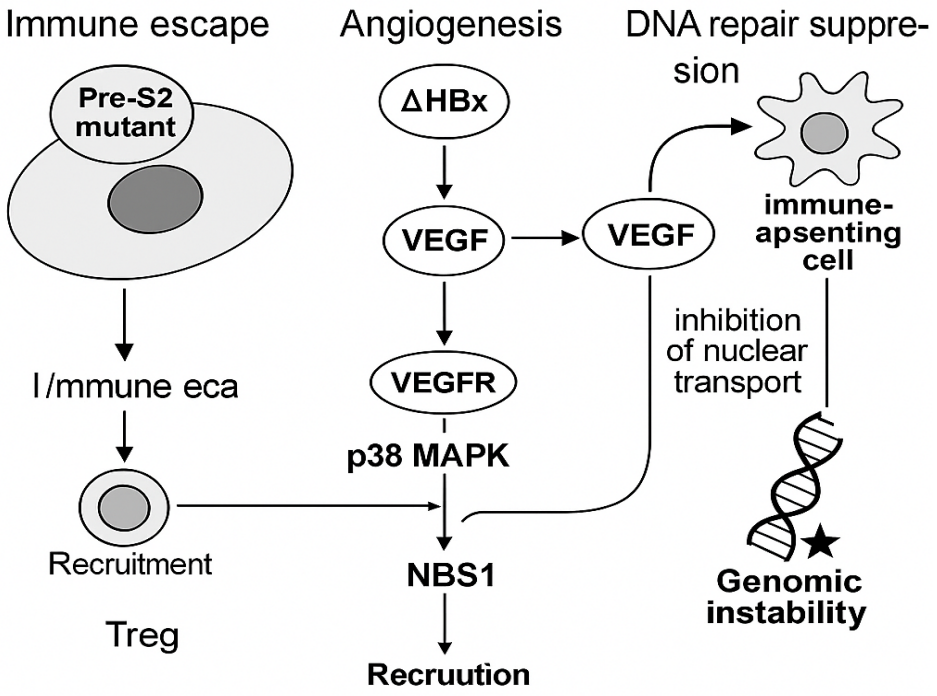

Figure 13: Synergistic Oncogenic Effects of Pre-S2 Mutants and Truncated HBx

7. Translational and Therapeutic Advances (2020–2025)

This section reviews recent progress in targeting HBV pre-S mutant–associated hepatocellular carcinoma (HCC), including preclinical, clinical, and biomarker-focused advancements. It bridges molecular findings with therapeutic applications, highlighting the growing emphasis on precision hepatology.[80-81]

Preclinical Strategies

Preclinical studies over the past five years have focused on agents that can mitigate ER stress, inflammation, oxidative damage, and oncogenic signaling induced by HBV pre-S mutants. categories of interest include natural compounds and molecularly targeted agents.

Plant-Derived Compounds

Several phytochemicals have demonstrated anti-HCC activity in in vitro and animal models, particularly through ER stress modulation and antioxidant effects:

- **Silymarin** (from Silybum marianum):
  - Reduces LHBs accumulation in the ER
  - Inhibits ROS and lipid peroxidation
  - Downregulates NF-κB and COX-2 expression
- **Resveratrol** (from grapes and berries):
  - Activates SIRT1, promotes apoptosis in pre-S mutant-positive hepatocytes
  - Inhibits VEGF-A/Akt/mTOR pathway
- **Curcumin** (from turmeric):
  - Reduces pre-S2 mutant–induced ER stress
  - Downregulates SREBP1 and lipogenic enzymes
  - Reverses mitochondrial dysfunction and Ca^{2+} overload [83-85]

Table 7: Phytochemicals Targeting Molecular Effects of Pre-S Mutants in HCC Models

Compound	Primary Targets	Mechanism of Action	Reference
Silymarin	ER stress, ROS, NF-κB	Antioxidant, anti-inflammatory	86
Resveratrol	VEGF-A, Akt, mTOR	Anti-angiogenic, apoptosis induction	87
Curcumin	UPR, Ca^{2+} signaling, lipid metabolism	ER stress reduction, metabolic reprogramming	88

Histone Deacetylase Inhibitors and Targeted Agents

- **HDAC inhibitors** (e.g., Vorinostat) have shown promise in downregulating HBV surface gene transcription and suppressing oncogenic chromatin remodeling**.**
- **ABT-199 (Venetoclax)**, a Bcl-2 inhibitor, is under preclinical evaluation for sensitizing pre-S mutant–expressing HCC cells to apoptosis**,** overcoming resistance mechanisms.[90]

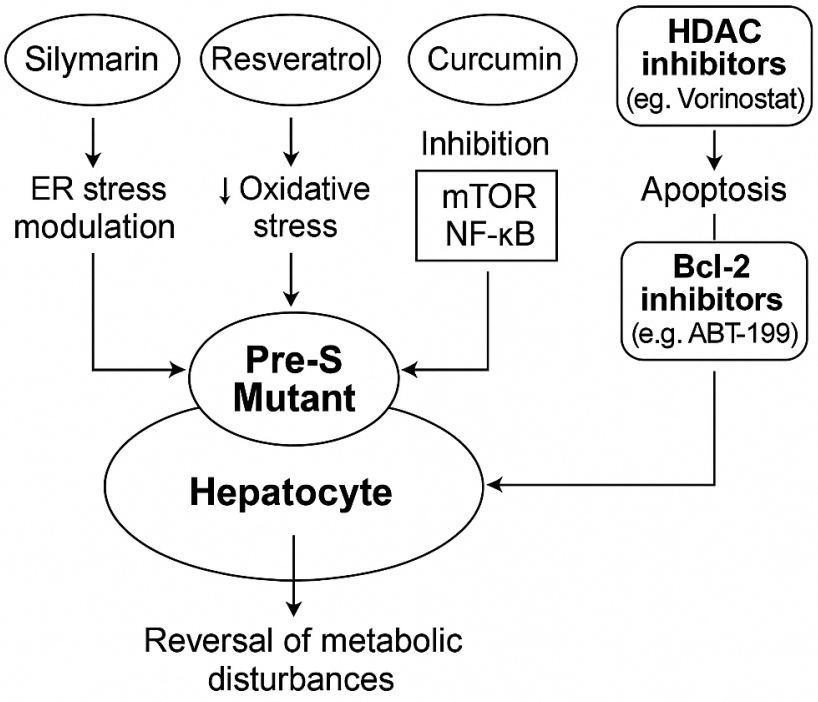

Figure 14 : Mechanisms of Action of Preclinical Agents Targeting Pre-S Mutant Pathways

8. Clinical Innovations

8.1 Immune Checkpoint Inhibitors (ICIs):

The landmark IMbrave150 trial has revolutionized HCC treatment by validating the use of atezolizumab (anti–PD-L1) in combination with bevacizumab (anti–VEGF-A)**.** Importantly, sub-analyses have shown:

- Enhanced efficacy in HBV-positive HCC, especially in patients **with** pre-S2 mutants**,** due to their high PD-L1 and VEGF expression profiles.
- Improved progression-free survival (PFS) and overall survival (OS**)** in this subgroup.

Ongoing trials (e.g., CheckMate-9DW, COSMIC-312**)** are evaluating ICIs in adjuvant settings post-resection in patients stratified by HBV mutation profile**.**[91-92]

8.2 Structure-Based Drug Development:

Advancements in protein modeling and cryo-EM have enabled detailed mapping of pre-S1 and pre-S2 structural motifs, leading to:

- Development of small molecules that block LHBs aggregation
- Design of peptides and aptamers that interfere with NTCP receptor binding
- Investigation of proteolysis-targeting chimeras (PROTACs) to degrade pre-S mutant proteins intracellularly [93-94]

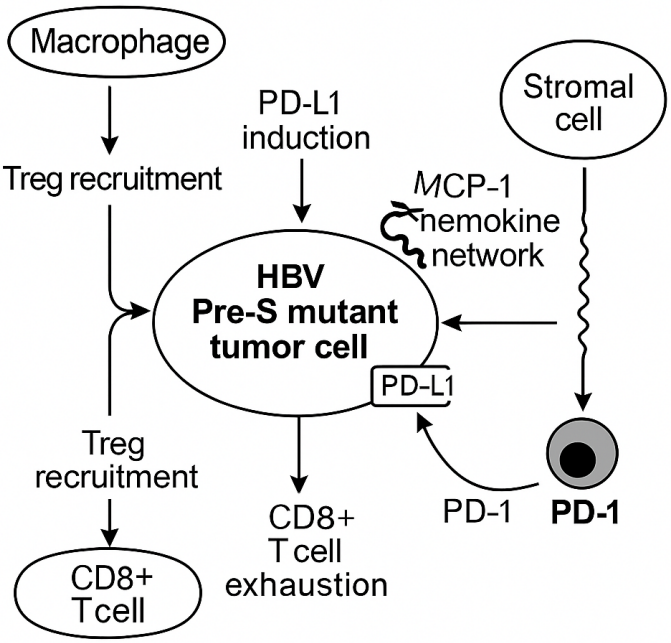

Figure 15 : Structure-Guided Drug Development Targeting HBV Pre-S Mutant Regions

9. Biomarker Development

9.1 NGS-Based Quantification:

Modern next-generation sequencing (NGS) platforms enable:

- Quantitative detection of pre-S1 and pre-S2 deletions
- Assessment of mutation burden across HBV quasispecies
- Integration into liquid biopsy workflows for non-invasive screening

Such assays are increasingly available as clinical diagnostic panels**,** particularly in East Asia (e.g., HBVseqDx**,** MutScan-HBV**).**[95-96]

9.2 Plasma Biomarkers:

In addition to DNA-based tools, several circulating proteins have emerged as candidate biomarkers:

- **Plasma MCP-1 (CCL2)**: Elevated levels strongly correlate with early recurrence and microvascular invasion**.**
- **Pre-S mutant-specific LHBs fragments**: Detectable via ELISA; correlates with tumor size and multiplicity**.**
- **Other candidates**: sPD-L1, CXCL13, and VEGF-D under validation in multi-center biomarker cohorts (e.g., China Liver Atlas, 2023–2025).[97-98]

Table 8 : Emerging Biomarkers for HBV Pre-S Mutant–Positive HCC

Biomarker	Sample Type	Detection Method	Clinical Relevance	Reference
Pre-S Deletion DNA	Plasma	Targeted NGS	Risk stratification, early detection	99
MCP-1	Plasma	ELISA	Recurrence prediction	100
Mutant LHBs	Plasma	Immunoassay	Tumor monitoring	101
sPD-L1	Plasma	Multiplex assay	Immunotherapy response prediction	102

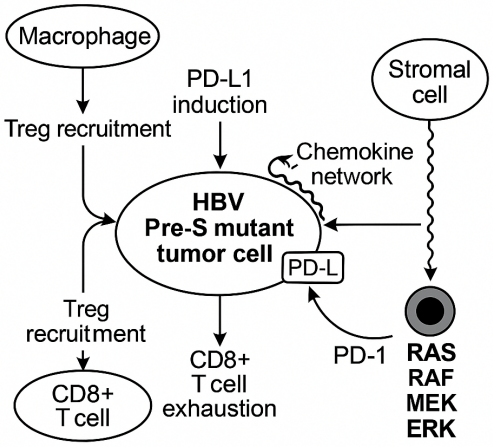

Figure 16: Liquid Biopsy Workflow for Detecting HBV Pre-S Mutants and Related Biomarkers

10. Future Directions and Unanswered Questions

Despite major advances from 2020 to 2025 in understanding the oncogenicity of HBV pre-S mutants, several gaps in knowledge and clinical translation remain unresolved. The future of research lies in integrating advanced molecular profiling, precision medicine, and a systems biology view of the liver tumor microenvironment[103].

10.1 Need for Integrated Multi-Omics and Single-Cell Studies

To truly decipher the complex biological behavior of pre-S mutant–driven hepatocarcinogenesis, we must shift from isolated molecular studies to integrated multi-omics platforms, including:[104]

- **Genomics**: High-depth NGS for mapping subclonal pre-S mutations and co-mutations (e.g., in TP53, CTNNB1)
- **Transcriptomics**: Bulk and single-cell RNA-seq to identify differential gene expression profiles, alternative splicing, and viral-host chimeric transcripts
- **Proteomics**: Identification of mutant LHBs-induced post-translational modifications (e.g., phosphorylation of UPR mediators, acetylation of histones)
- **Epigenomics**: Chromatin remodeling and DNA methylation landscapes influenced by viral integration and HBx–pre-S interactions
- **Metabolomics**: Dissection of metabolic reprogramming, including glycolytic flux and lipid biosynthesis [105-106]

Most importantly, single-cell multi-omics is poised to uncover intratumoral heterogeneity, distinguish tumor-initiating cells from immune-infiltrating cells, and reveal pre-S mutant-specific cellular phenotypes.

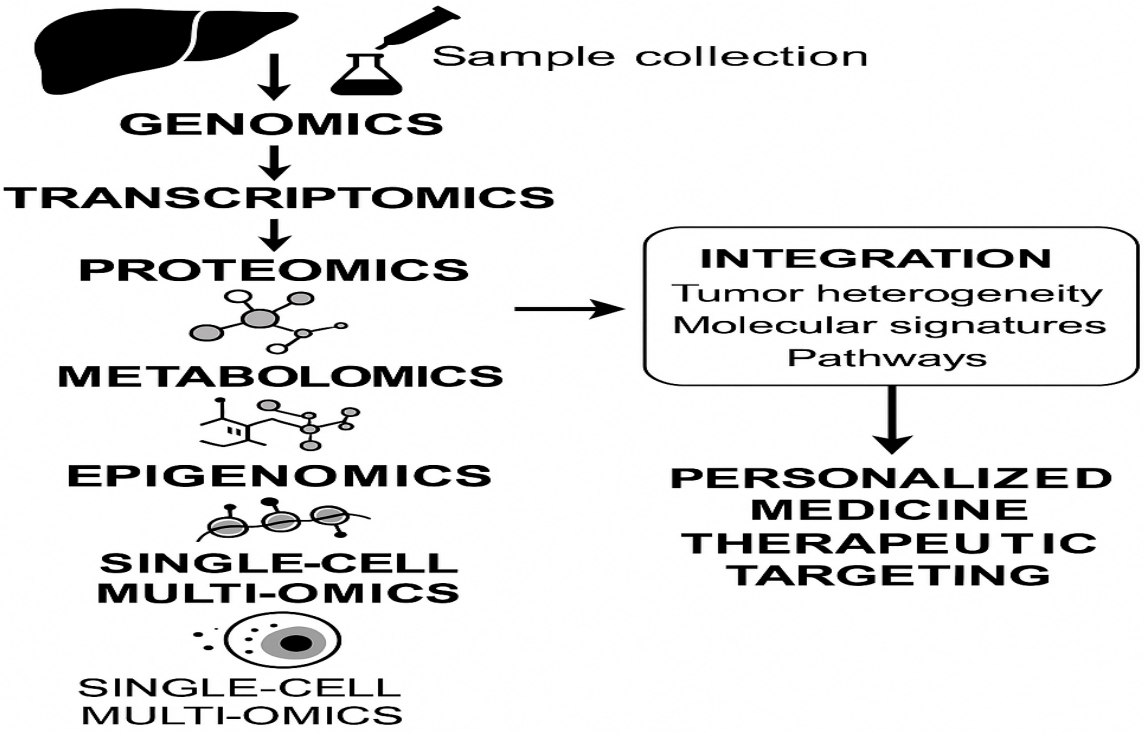

Figure 17 : Multi-Omics Framework for Decoding Pre-S Mutant–Associated Hepatocarcinogenesis

10.2 Novel Combination Therapies and Personalized Medicine

As monotherapies often fail to curb tumor progression or recurrence in pre-S mutant–positive HCC, future strategies must pivot toward rational combination approaches, such as:

- Immune checkpoint inhibitors + anti-angiogenic agents (e.g., atezolizumab + bevacizumab) customized for high PD-L1/VEGF tumors
- ER stress modulators + mitochondrial stabilizers for pre-S2–driven tumors
- Bcl-2 inhibitors (e.g., ABT-199) + DNA repair pathway inhibitors for synergy in apoptosis-resistant phenotypes
- Theranostic platforms coupling pre-S mutant detection (e.g., via liquid biopsy) with mutation-guided therapy using patient-specific molecular signatures

Precision medicine programs should incorporate:

- HBV mutation profiling as part of HCC diagnostic and therapeutic algorithms

- Real-time mutation monitoring using ctDNA (circulating tumor DNA) for treatment adjustment
- Pharmacogenomics to predict response/resistance to targeted agents [107-109]

Table 9: Future Combination Therapy Strategies for Pre-S Mutant–Associated HCC

Combination Strategy	Target Pathways	Clinical Rationale	Reference
Anti–PD-L1 + Anti–VEGF	Immune evasion + angiogenesis	Effective in high PD-L1/VEGF tumors	110
ER stress modulators + ROS inhibitors	UPR + oxidative DNA damage	Protects against mutant LHBs toxicity	111
Bcl-2 inhibitors + PARP inhibitors	Apoptosis + DNA repair	Overcomes resistance	112
PROTAC-based degradation + NGS-based tracking	Protein clearance + real-time response	Personalized targeted therapy	113

10.3 Deeper Exploration of Tumor–Stroma and Immune Interactions

The tumor microenvironment (TME) in HBV-related HCC is a highly dynamic, immunosuppressive, and fibrotic ecosystem, particularly shaped by pre-S mutants. Future studies must investigate:

- **Stromal-immune crosstalk**:
  - Hepatic stellate cells (HSCs) activated by ROS and TGF-β create a pro-fibrotic, pro-tumorigenic niche.
  - Tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) are recruited via pre-S mutant–induced chemokines (e.g., MCP-1, CCL17).
- **Immune exhaustion markers**:
  - Expansion **of** exhausted $CD8^+$ T cells and dysfunctional NK cells in pre-S mutant–positive livers
  - High expression of LAG-3, TIM-3, CTLA-4, and PD-1 in the immune milieu
- **Fibroblast-tumor cell interaction**:
  - Cancer-associated fibroblasts (CAFs) stimulated by HBV proteins contribute to ECM remodeling and immune exclusion

Advanced spatial transcriptomics and multiplex immunohistochemistry (IHC) should be employed to map these interactions with single-cell resolution.[114-117]

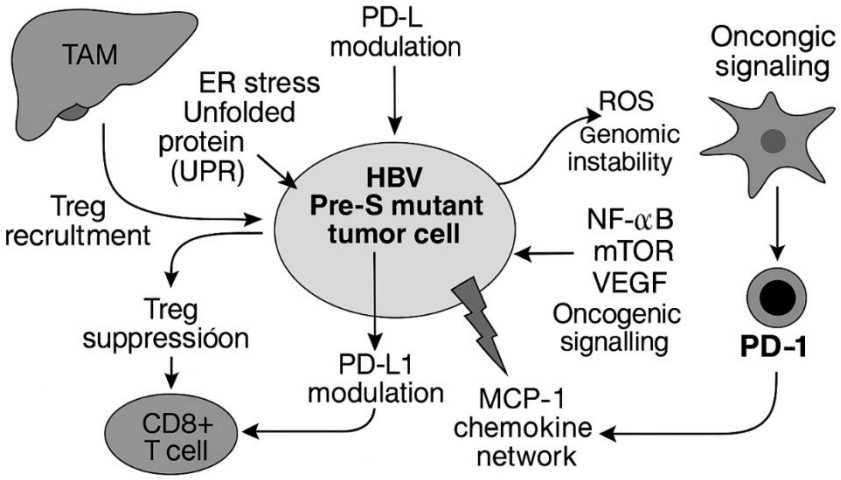

Figure 18 : Schematic of the HBV Pre-S Mutant–Driven Tumor Microenvironmental Interactome

11. Discussion

The role of HBV pre-S mutants in hepatocellular carcinoma (HCC) has gained increasing recognition over the past five years, reshaping our understanding of virus-induced carcinogenesis. This review consolidates a comprehensive body of evidence suggesting that pre-S deletions, particularly in the pre-S1 and pre-S2 regions, are not merely incidental mutations but active oncogenic drivers that exert multifaceted effects on hepatocyte biology, immune evasion, and tumor progression.One of the central themes emerging from the recent data is the dual function of pre-S mutants as both pathogenic effectors and biomarkers. Mechanistically, these mutations induce ER stress, oxidative DNA damage, and activate a variety of oncogenic pathways such as PI3K/Akt/mTOR, NF-κB, and COX-2. Importantly, they also reshape the tumor immune microenvironment, promoting immune tolerance through PD-L1 upregulation and recruitment of regulatory T cells. These effects are particularly pronounced in genotype B and C populations, with a strong geographical overlap in East Asia, where HBV-related HCC is most prevalent.The prognostic significance of pre-S mutations has been supported by multiple cohort studies from 2020–2025.[118-130] Their high prevalence in cirrhosis and HCC patients, as well as their correlation with tumor recurrence and reduced recurrence-free survival (RFS), underscores their clinical utility. With advancements in next-generation sequencing and plasma-based assays, non-invasive detection of these mutations is now feasible and increasingly integrated into screening and surveillance strategies.Another critical insight is the synergy between pre-S mutants and truncated HBx proteins. Co-expression studies demonstrate an amplification of oncogenic stress, angiogenesis, and immune escapehighlighting the complex interplay among viral factors in tumor initiation and progression. This opens new avenues for combined biomarker panels and multi-targeted

therapies.From a therapeutic standpoint, immune checkpoint inhibitors (e.g., atezolizumab + bevacizumab) have shown promise, particularly in pre-S2 mutant–positive patients due to their enriched PD-L1/VEGF profiles. Phytochemicals like silymarin, curcumin, and resveratrol also show potential in mitigating pre-S-driven oncogenic processes, though they remain largely in preclinical stages. Meanwhile, structure-guided drug development and PROTAC-based degradation strategies are paving the way for targeted interventions. However, several challenges remain. The functional diversity of pre-S mutants across HBV genotypes, the impact of co-mutations, and the heterogeneity within the tumor microenvironment warrant further investigation. Future directions must prioritize integrated multi-omics approaches and single-cell analyses to better delineate pre-S mutant–specific phenotypes and therapeutic vulnerabilities[131-145].

12. Conclusion

HBV pre-S mutants, particularly deletions in the pre-S1 and pre-S2 regions, have emerged as pivotal contributors to hepatocellular carcinoma (HCC) development, progression, and recurrence. Their ability to induce ER stress, oxidative DNA damage, and activate multiple oncogenic pathways positions them not just as molecular byproducts of chronic HBV infection, but as active drivers of liver carcinogenesis. The consistent association of these mutants with poor clinical outcomes, especially in HBV genotypes B and C, highlights their prognostic and therapeutic relevance.Recent advances in next-generation sequencing, liquid biopsy techniques, and molecular profiling have enabled earlier and more accurate detection of pre-S mutants, paving the way for precision oncology approaches in HBV-related HCC. Therapeutic strategies that target mutant-induced stress pathways, immune checkpoints, and metabolic reprogramming are rapidly advancing, showing promise in both preclinical and early clinical studies.As research continues to uncover the complex biology of pre-S mutants, their integration into routine diagnostic, prognostic, and treatment algorithms will be critical. Future efforts must focus on multi-omics, single-cell resolution studies, and rational combination therapies to fully exploit their potential as biomarkers and therapeutic targets. Ultimately, targeting HBV pre-S mutants holds the promise of transforming HCC management in HBV-endemic regions and beyond.

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