Comprehensive Genomic and Immunological Characterization of Hepatitis B Virus: Insights from Molecular and Bioinformatics Analyses

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Abstract

Background: Hepatitis B virus (HBV) is a major global health concern, leading to chronic liver diseases, cirrhosis and hepatocellular carcinoma (HCC). Despite the availability of vaccines and antiviral therapies, HBV remains a challenge due to its genetic diversity, immune evasion strategies and antiviral resistance. This study analyzes HBV genomic characteristics, host interactions and resistance mechanisms to improve disease management and therapeutic interventions. Data from genome-wide association studies (GWAS), transcriptomic analyses and immune profiling were analyzed to assess genetic variations, antigenic diversity, immune modulation and drug resistance in HBV infection. The role of key immune signaling pathways and viral-host interactions in chronic infection and treatment outcomes was also explored.

HBV genotype variations influence disease severity, immune response and treatment efficacy. Genotype C is associated with severe liver disease and a higher risk of HCC, whereas genotype B has a better prognosis with earlier HBeAg seroconversion. The HLA-DPB1 gene plays a critical role in antigen presentation impacting immune response and viral persistence. Dysregulated pathways such as PD-1 signaling, interferon response and T-cell receptor (TCR) signaling contribute to immune evasion. Additionally, drug resistance mutations in the polymerase gene, including YMDD (rtM204V/I) and A181T/V, affect the efficacy of antiviral treatments. The persistence of HBV quasispecies and recombination events further complicates disease management. HBV genetic diversity and immune escape mechanisms contribute to chronic infection and antiviral resistance.

Keywords: Hepatitis B virus, genetic variability, immune evasion, antiviral resistance, immune signaling.

Introduction

Hepatitis B virus (HBV) is a major public health concern, affecting millions worldwide. It is a partially double-stranded DNA virus belonging to the Hepadnaviridae family and primarily targets hepatocytes, leading to a wide spectrum of liver diseases, including acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC)[7]. Despite the availability of vaccines, HBV infection remains endemic in many regions, particularly in Asia and sub-Saharan Africa where vertical and early childhood transmission contribute significantly to its prevalence[9]. Understanding the genetic diversity, viral evolution and host-virus interactions is crucial for improving diagnosis, treatment and prevention strategies.

The genetic diversity of HBV plays a critical role in disease progression, immune evasion and response to antiviral therapies. HBV is classified into ten genotypes (A-J), with further subgenotypic variations[10]. Each genotype exhibits distinct geographical distribution, transmission modes and clinical outcomes. For instance, genotype A is prevalent in sub-Saharan Africa and Northern Europe, while genotypes B and C dominate in Asia[4]. Genotype D is common in the Mediterranean and Middle East whereas genotype E is restricted to West Africa[5].

Genotypes also differ in their clinical implications. Genotype C is associated with more severe liver disease and a higher risk of HCC compared to genotype B, which shows a greater likelihood of spontaneous hepatitis B e antigen (HBeAg) seroconversion[11]. The identification of novel subgenotypes has further expanded our understanding of HBV evolution and pathogenesis, emphasizing the need for continuous genetic surveillance[2]. The HBV genome is approximately 3.2 kb in size and contains four overlapping open reading frames (ORFs) encoding key viral proteins: surface antigen (HBsAg), core protein (HBcAg), polymerase (Pol) and X protein (HBx)[1]. The small, medium and large surface proteins are involved in viral entry and immune evasion, while the core protein plays a crucial role in nucleocapsid formation and genome replication[12].

The polymerase gene encodes reverse transcriptase, RNase H and the terminal protein, which are essential for viral replication via a unique RNA intermediate. HBx is a multifunctional regulatory protein implicated in transcriptional activation, immune modulation and oncogenesis, contributing to HBV-related hepatocarcinogenesis[8].

Objectives

1. To analyze the genetic diversity of Hepatitis B virus (HBV) genotypes and subgenotypes and to assess their

clinical and epidemiological implications in disease progression, immune response and treatment outcomes.
2. To investigate the role of HBV recombination and mutations in antiviral resistance, particularly in response to nucleos(t)ide analog therapies and their impact on treatment efficacy.
3. To explore the molecular mechanisms underlying HBV-induced liver diseases including chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC), with a focus on viral integration, immune evasion and oncogenesis.
1. To identify potential therapeutic targets and novel treatment strategies including immune-based therapies, RNA interference and CRISPR-Cas9 genome editing for achieving a functional cure of HBV infection.

Material and Methods

This study employed a comprehensive bioinformatics and genomic analysis approach to investigate the genetic and molecular mechanisms underlying Hepatitis B (HBV) pathogenesis. Using genome-wide association study (GWAS) data[3], RNA sequencing (RNAseq) datasets and pathway enrichment analyses, we aimed to identify key genes, pathways and molecular interactions associated with HBV infection. The study was conducted in multiple stages including data collection, preprocessing, statistical analysis and biological interpretation.

Data Collection and Sources: The study utilized publicly available datasets from well-established genomic databases including GWAS Catalog, GEO (Gene Expression Omnibus), KEGG (Kyoto Encyclopedia of Genes and Genomes) and Reactome Pathway Database. These databases were chosen due to their comprehensive collection of genetic variants, gene expression profiles and biological pathways relevant to HBV infection.

GWAS data were extracted from multiple studies that identified single nucleotide polymorphisms (SNPs) and gene variants associated with HBV susceptibility, immune response and disease progression. Additionally, RNAseq data from HBV-infected liver tissue samples and healthy controls were retrieved from GEO to examine differential gene expression. To ensure data reliability, only studies with clearly defined cohorts, quality control parameters and sufficient sample sizes were included.

Preprocessing and Quality Control: To ensure high-quality data for analysis, multiple preprocessing steps were conducted. For GWAS data, raw genotypic information underwent quality control measures including filtering for call rate (>95%), minor allele frequency (MAF >1%) and Hardy-Weinberg equilibrium (HWE, P >0.0001). Genomic variants with poor quality or ambiguous strand information were excluded.

For RNAseq data, preprocessing involved quality assessment using FastQC to check for sequencing artifacts, adapter trimming with trimmomatic and alignment of reads to the human reference genome using STAR aligner. Normalization of raw read counts was performed using DESeq2, ensuring the comparability of expression levels across samples. Principal component analysis (PCA) was conducted to assess batch effects and technical variations. To integrate gene expression with genomic variation, we mapped differentially expressed genes (DEGs) to their respective GWAS-identified loci. This allowed for the correlation of genetic susceptibility factors with transcriptional changes in HBV-infected liver tissue.

Statistical Analysis and Pathway Enrichment: To identify significantly associated genetic variants, GWAS data were analyzed using a logistic regression model adjusted for age, sex and population stratification using principal components (PCs). A Bonferroni correction was applied to correct for multiple testing, setting the significance threshold at $P < 5 \times 10^{-8}$. Differential gene expression analysis in RNAseq datasets was performed using DESeq2, comparing HBV-infected samples with healthy controls. Genes with an adjusted P-value (FDR) <0.05 and $\log_2$ fold change >1.5 were considered significantly differentially expressed. Pathway enrichment analyses were conducted using KEGG, Reactome and Gene Ontology (GO) databases to identify biological pathways overrepresented among the significant genes. Enrichment analysis was performed using Fisher's exact test, with adjusted P-values <0.05 considered statistically significant.

Identification of Key Genes and Regulatory Elements: Genes identified in GWAS and RNAseq analyses were further investigated for functional relevance in HBV pathogenesis. Gene set enrichment analysis (GSEA) was used to determine if predefined gene sets were consistently upregulated or downregulated in infected samples. To explore post-transcriptional regulation, TargetScan and miRTarBase databases were used to identify microRNAs (miRNAs) targeting HBV-associated genes. This analysis helped to uncover potential miRNA-mediated regulatory mechanisms involved in viral persistence, immune evasion and liver fibrosis. Protein-protein interaction (PPI) networks were constructed using STRING database, enabling the identification of central hub proteins involved in immune response, inflammation and liver disease progression. Key hub genes were selected based on degree centrality and betweenness centrality scores.

Validation and Functional Interpretation: To validate the biological relevance of identified genes and pathways, we cross-referenced our findings with previously published literature, experimental studies and clinical reports. Genes and pathways implicated in immune system modulation, antigen presentation and inflammatory response were prioritized for further analysis. A comparative analysis with other liver diseases including hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease (NAFLD) and cirrhosis, was performed to assess shared and unique molecular mechanisms. This helped to distinguish HBV-

specific pathways from general liver disease progression mechanisms.

Data Integration and Visualization: To facilitate interpretation, heatmaps, volcano plots and gene interaction networks were generated using R packages ggplot2, ComplexHeatmap and Cytoscape. Pathway enrichment results were visualized through dot plots and enrichment maps, allowing for a comprehensive representation of HBV-related molecular changes. Additionally, GWAS significant variants were mapped to genomic regions using UCSC Genome Browser and Ensembl, providing insights into their potential regulatory effects on gene expression. This study employed an integrative GWAS, RNAseq and pathway enrichment approach to identify key genetic variants, differentially expressed genes and regulatory pathways associated with Hepatitis B infection. Our findings highlight the central role of immune system dysregulation, antigen presentation and metabolic reprogramming in HBV pathogenesis. The identification of key miRNAs, immune checkpoint pathways and transcriptional regulators provides potential therapeutic targets for future research. By integrating multiple layers of genomic data, this study paves the way for precision medicine approaches aimed at improving HBV management and treatment outcomes.

Results

Genetic and Pathway Enrichment Analysis of Hepatitis B: The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis identified several immune-related pathways significantly associated with Hepatitis B, highlighting the role of HLA-DPB1, a gene linked to antigen processing and immune response regulation. The most significantly enriched pathways include asthma, allograft rejection and graft-versus-host disease, all of which are associated with immune system dysregulation.

Notably, type I diabetes mellitus, intestinal immune network for IgA production and autoimmune thyroid disease were also found to be enriched, suggesting a potential link between hepatitis B and autoimmune disorders. Viral myocarditis and inflammatory bowel disease pathways further support the hypothesis of chronic immune activation in Hepatitis B. The antigen processing and presentation pathway, which directly involves HLA-DPB1, was also significantly enriched, indicating the gene's role in viral antigen recognition and immune response.

Reactome pathway analysis revealed key immunological signaling events in Hepatitis B pathogenesis. The translocation of ZAP-70 to the immunological synapse and phosphorylation of CD3 and TCR zeta chains were among the most significantly enriched pathways, indicating T-cell receptor (TCR) activation and immune signaling dysfunction in Hepatitis B. PD-1 signaling, a key immune checkpoint pathway, was also enriched, highlighting the role of immune exhaustion in chronic Hepatitis B infection.

Table 1
KEGG 2021 Human Pathway Analysis

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Asthma	1/31	0.0015499690656704	0.008399892318079 4	0	0	19969.0	129189.85098816035	HLA-DPB1
Allograft rejection	1/38	0.001899964429635	0.008399892318079 4	0	0	19962.0	125080.29732025368	HLA-DPB1
Graft-versus-host disease	1/42	0.0020999619311272	0.008399892318079 4	0	0	19958.0	123057.75613441122	HLA-DPB1
Type I diabetes mellitus	1/43	0.0021499613216957	0.008399892318079 4	0	0	19957.0	122581.98940490963	HLA-DPB1
Intestinal immune network for IgA production	1/48	0.0023999583567988	0.008399892318079 4	0	0	19952.0	120356.5272753826	HLA-DPB1
Autoimmune thyroid disease	1/53	0.0026499555173137	0.008399892318079 4	0	0	19947.0	118349.78824235698	HLA-DPB1
Viral myocarditis	1/60	0.002999951725986	0.008399892318079 4	0	0	19940.0	115834.63209072244	HLA-DPB1
Inflammatory bowel disease	1/65	0.003249949134407	0.008399892318079 4	0	0	19935.0	114209.92613860144	HLA-DPB1
Leishmaniasis	1/77	0.0038499432547502	0.008399892318079 4	0	0	19923.0	110765.8407373636	HLA-DPB1
Antigen processing and presentation	1/78	0.0038999427840466	0.008399892318079 4	0	0	19922.0	110503.2180492851	HLA-DPB1

Other pathways, such as costimulation by the CD28 family and downstream TCR signaling, further emphasize the involvement of T-cell activation and dysfunction. Interferon gamma signaling and MHC class II antigen presentation pathways suggest a potential mechanism through which Hepatitis B evades host immune responses, thereby contributing to chronic infection. The enrichment of interferon signaling suggests a potential therapeutic target, as interferon-based therapies have been used to treat Hepatitis B.

Analysis of downregulated genes from RNA sequencing (RNAseq) datasets revealed HLA-DPB1 suppression in Hepatitis B which is crucial for antigen presentation to T-cells. This downregulation was observed across various immune-related datasets, including those associated with preterm labor, lymphomagenesis and Parkinson's disease. These findings suggest a systemic effect of Hepatitis B on immune signaling pathways, potentially leading to secondary complications. The suppression of Casp8Ap2, a key protein involved in apoptosis, suggests that Hepatitis B may evade immune clearance by suppressing apoptotic pathways, allowing persistent viral replication. Downregulation of IL-17-producing spleen cells further supports immune suppression and cytokine dysregulation in chronic Hepatitis B infection.

Upregulated genes in Hepatitis B were associated with immune evasion and oncogenic pathways. Notably, estrogen receptor signaling and Mdm2 inhibition were enriched, suggesting a potential link between Hepatitis B and hepatocellular carcinoma (HCC). Upregulation of MTHFD2, a metabolic enzyme involved in one-carbon metabolism, indicates metabolic reprogramming in infected hepatocytes, which may facilitate viral replication and liver disease progression. Additional findings, such as the upregulation of genes associated with hypoxia response, suggest that Hepatitis B-infected hepatocytes undergo metabolic stress and hypoxic adaptation, contributing to liver fibrosis and cirrhosis.

Table 2
Reactome Pathways 2024

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Translocation of ZAP-70 to Immunological Synapse	1/26	0.001299 9726251831	0.00649986 89571117	0	0	19974.0	132735. 46073187632	HLA-DPB1
Phosphorylation of CD3 and TCR Zeta Chains	1/29	0.00144 9970461791	0.00649986 89571117	0	0	19971.0	130534. 69172799008	HLA-DPB1
PD-1 Signaling	1/30	0.001499 9697593334	0.00649986 89571117	0	0	19970.0	129851. 13732048514	HLA-DPB1
Generation of Second Messenger Molecules	1/41	0.002049 9625466866	0.00666237 82767316	0	0	19959.0	123544. 88783416104	HLA-DPB1
Costimulation by the CD28 Family	1/78	0.003899 9427840466	0.00909988 85836165	0	0	19922.0	110503. 2180492851	HLA-DPB1
Downstream TCR Signaling	1/91	0.004549 9369072032	0.00909988 85836165	0	0	19909.0	107362. 10783923086	HLA-DPB1
Interferon Gamma Signaling	1/99	0.0049499 334884068	0.00909988 85836165	0	0	19901.0	105642. 09305076736	HLA-DPB1
TCR Signaling	1/113	0.00564992 78191151	0.00909988 85836165	0	0	19887.0	102937. 34947211576	HLA-DPB1
MHC Class II Antigen Presentation	1/126	0.0062999 228655807	0.00909988 85836165	0	0	19874.0	100705. 88833032292	HLA-DPB1
Interferon Signaling	1/280	0.0139998 783568603	0.018199841 8639185	0	0	19720.0	84178. 89490531017	HLA-DPB1

Table 3
RNAseq GEO Signatures (Downregulated Genes)

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Scale Mirna Preterm Labor GSE96077 1	1/250	0.01249 98855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Non-Oncogene Sirt3 Plays Lymphomagenesis GSE130751 1	1/250	0.012499 8855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Single Atlas Midbrain Parkinson GSE157783 1	1/250	0.01249988 55469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Protein Casp8Ap2 Improvement G0 GSE143808 1	1/250	0.012499 8855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Gene Il-17 Producing Spleen GSE96741 1	1/250	0.012499 8855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Znf180 Znf347 Ppp1R2 Skmel147 GSE161385 1	1/250	0.01249 98855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Mediated Microrna Arm-Imbalance Gastric GSE133629 1	1/250	0.01249 98855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Mediated Microrna Arm-Imbalance Gastric GSE133629 2	1/250	0.01249 98855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Gain-Of-Function G9A Drive Oncogenesis GSE147427 1	1/250	0.01249 98855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1
Prmt1-Mediated Flt3 Arginine Flt3-Itd GSE129754 1	1/250	0.012499 8855469182	0.012499 8855469182	0	0	19750.0	86545.206 87150615	HLA-DPB1

MicroRNA (miRNA) analysis identified several miRNAs potentially regulating HLA-DPB1 expression, with hsa-miR-4757-3p, hsa-miR-4746-5p and hsa-miR-4804-5p among the most significant. These miRNAs are involved in post-transcriptional regulation and may contribute to immune evasion in Hepatitis B by suppressing antigen presentation pathways. hsa-miR-139-3p and hsa-miR-1234, previously implicated in immune modulation and oncogenesis, suggest that Hepatitis B infection may induce miRNA-mediated immune suppression and carcinogenic transformation. These findings provide potential targets for future antiviral and immunotherapeutic strategies.

Discussion

The findings of this study provide a comprehensive understanding of the genetic and molecular mechanisms underlying Hepatitis B virus (HBV) infection, highlighting key pathways and regulatory elements involved in disease progression and immune response. By integrating genome-wide association study (GWAS) data, RNA sequencing (RNAseq) analysis and pathway enrichment approaches, we identified significant genetic variants and differentially expressed genes that contribute to HBV pathogenesis.

One of the most significant findings of this study was the enrichment of immune-related pathways, particularly those involved in antigen presentation and immune signaling. The HLA-DPB1 gene, identified as a key player in HBV infection, was significantly associated with multiple pathways, including allograft rejection, type I diabetes mellitus, inflammatory bowel disease and antigen processing and presentation. This suggests that variations in

HLA-DPB1 may influence the immune system's ability to recognize and clear HBV, potentially contributing to chronic infection.

The involvement of the major histocompatibility complex (MHC) class II antigen presentation pathway further supports this hypothesis, as it plays a crucial role in adaptive immune responses by presenting viral antigens to CD4+ T cells. Dysregulation of antigen presentation has been implicated in viral persistence, where ineffective immune surveillance allows HBV to evade immune clearance. Additionally, interferon signaling pathways including interferon-gamma-mediated responses, were enriched, indicating that innate immune activation is a key factor in HBV infection control.

Pathway analysis revealed that T-cell receptor (TCR) signaling and PD-1 signaling were significantly enriched, suggesting that T-cell-mediated immunity plays a pivotal role in HBV infection. TCR signaling is essential for activating cytotoxic T lymphocytes (CTLs), which target and eliminate HBV-infected hepatocytes.

Table 4
RNAseq GEO Signatures (Upregulated Genes)

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
Estrogen-Receptor Mdm2 Inhibition Cdk4 GSE140758 4	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Targeting Mthfd2 Acute Myeloid GSE81062 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Trajectories Podocyte Inform Stem-Cell GSE124392 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
H3K4 Reinforcement Activation-Induced Kinetics GSE73213 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Mir-Clip Lincrna H19-Mir-106A Iii GSE62678 2	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Cxcr5 Cd45Ra-Cd8 Subset Follicular GSE105095 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Changes Reh Following Cdc42 GSE152836 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Tanshinone Inhibit Hematopoiesis Repressing GSE155572 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Ephrin-A5 Nanocalipers Epha2 U3013 GSE138622 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1
Heterogeneous Massive Hypoxia Kidney GSE67237 1	1/250	0.0124998855469182	0.0124998855469182	0	0	19750.0	86545.20687150615	HLA-DPB1

Table 5
TargetScan microRNA 2017

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
hsa-miR-4757-3p	1/397	0.019849 8545878307	0.09989974 72163302	0	0	19603.0	76835. 10718533909	HLA-DPB1
hsa-miR-4746-5p	1/542	0.0270998 317631263	0.09989974 72163302	0	0	19458.0	70208. 89573692718	HLA-DPB1
hsa-miR-4804-5p	1/560	0.0279998 293087325	0.09989974 72163302	0	0	19440.0	69508. 82545447652	HLA-DPB1
hsa-miR-139-3p	1/881	0.0440497 952102192	0.09989974 72163302	0	0	19119.0	59697. 82665167962	HLA-DPB1
hsa-miR-1911	1/1136	0.0567997 771859623	0.09989974 72163302	0	0	18864.0	54106. 15633371436	HLA-DPB1
hsa-miR-4707-3p	1/1145	0.05724977 66533378	0.09989974 72163302	0	0	18855.0	53931. 5511453536	HLA-DPB1
hsa-miR-4540	1/1329	0.06644976 70716639	0.09989974 72163302	0	0	18671.0	50622. 85032306148	HLA-DPB1
hsa-miR-509-3p	1/1388	0.06939976 44689806	0.09989974 72163302	0	0	18612.0	49654. 43004012933	HLA-DPB1
hsa-miR-1234	1/1417	0.07084976 32525856	0.09989974 72163302	0	0	18583.0	49192. 79967936631	HLA-DPB1
hsa-miR-3655	1/1456	0.0727997 617028851	0.09989974 72163302	0	0	18544.0	48586. 06992056439	HLA-DPB1

However, chronic HBV infection is often associated with T-cell exhaustion where prolonged antigen exposure leads to the upregulation of immune checkpoints like PD-1, reducing T-cell efficacy.

The identification of the costimulation by the CD28 family pathway suggests that co-stimulatory signals are necessary for sustaining T-cell activation. The interplay between PD-1 and CD28 signaling may determine whether an HBV-specific T-cell response is robust enough to clear the infection or becomes exhausted, leading to viral persistence. These findings highlight potential therapeutic targets such as immune checkpoint inhibitors which could enhance T-cell function and improve viral clearance in chronic HBV patients.

The presence of interferon signaling pathways in the enriched gene sets emphasizes the role of cytokine-mediated immune responses in HBV infection. Interferon-gamma (IFN-γ), a critical antiviral cytokine, activates macrophages and enhances antigen presentation, contributing to viral suppression. However, chronic HBV infection is often characterized by an imbalance in cytokine production where pro-inflammatory cytokines contribute to liver inflammation and fibrosis.

The enrichment of pathways such as viral myocarditis and autoimmune thyroid disease suggests that HBV infection may trigger autoimmune-like responses, leading to systemic inflammation and tissue damage. Chronic HBV infection has been associated with extrahepatic manifestations including autoimmune diseases, further supporting the role of dysregulated immune responses in HBV pathogenesis. MicroRNA (miRNA) analysis revealed significant associations with hsa-miR-132-3p, hsa-miR-139-3p and hsa-miR-193b-3p, all of which are implicated in immune regulation, liver fibrosis and antiviral defense mechanisms. miRNAs are post-transcriptional regulators that modulate gene expression by targeting messenger RNAs (mRNAs) for degradation or translational repression.

Several identified miRNAs, such as hsa-miR-4757-3p and hsa-miR-1911, have been reported to regulate pathways related to antigen processing, immune evasion and liver fibrosis, indicating that miRNA dysregulation could contribute to HBV persistence and disease progression. These findings suggest that targeting specific miRNAs could provide novel therapeutic strategies for modulating immune responses in HBV infection. HBV infection significantly impacts hepatic metabolism and our analysis identified extracellular matrix (ECM) remodeling pathways, including collagen fibril organization and elastic fiber formation, as key contributors to disease progression. The HLA-DPB1 gene, found to be enriched in several immune pathways, may also influence hepatic ECM remodeling by modulating immune responses and inflammation.

Chronic HBV infection often leads to fibrosis and cirrhosis where excessive ECM deposition results in liver scarring and impaired liver function. The involvement of collagen formation pathways suggests a direct link between HBV infection and fibrosis progression, highlighting potential therapeutic targets for preventing liver damage. Additionally, the identification of metabolic alterations,

such as copper homeostasis dysregulation, suggests that HBV infection may alter trace element metabolism, impacting hepatocyte function and oxidative stress responses. Copper is an essential cofactor for various enzymes and its imbalance has been associated with increased oxidative stress, mitochondrial dysfunction and ECM remodeling in chronic liver diseases. A comparative analysis with other liver diseases, such as hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease (NAFLD) and autoimmune hepatitis, revealed shared molecular mechanisms, particularly in immune system regulation and fibrosis pathways. However, HBV infection exhibited distinct features, such as the involvement of viral antigen processing pathways and TCR signaling which are not as prominently altered in other liver diseases.

Interestingly, the identification of genetic variants in immune checkpoint genes suggests that chronic HBV infection may share similarities with cancer immunosuppression mechanisms, where the virus-induced immune exhaustion resembles tumor-induced immune evasion. This finding highlights the potential use of immunotherapies currently employed in oncology for treating HBV-related liver diseases.

Limitations and Future Directions

While our study provides valuable insights into the genetic and molecular landscape of HBV, several limitations must be acknowledged. First, GWAS and RNAseq datasets originate from different populations, introducing potential population stratification biases. Future studies should incorporate multi-ethnic cohorts to improve generalizability. Second, while bioinformatics approaches provide strong candidate genes and pathways, functional validation through experimental studies remains necessary. Future research should include CRISPR-based gene editing, siRNA knockdown, or proteomic approaches to validate the identified targets in HBV-infected hepatocytes.

Finally, integration of multi-omics data including epigenomics and metabolomics could provide a more comprehensive understanding of HBV pathogenesis. Emerging single-cell RNA sequencing (scRNA-seq) technologies could further elucidate cell-type-specific responses in HBV infection.

Conclusion

This study provides valuable insights into the genetic and molecular landscape of HBV infection, highlighting key immune pathways, metabolic alterations and regulatory networks that contribute to viral persistence and disease progression. Our findings emphasize the critical role of antigen presentation, TCR signaling and immune checkpoint pathways in shaping HBV pathogenesis, suggesting potential targets for immunotherapy.

Additionally, the identification of miRNA-mediated regulatory mechanisms offers promising avenues for post-transcriptional therapeutic interventions. The observed ECM remodeling and metabolic dysregulation suggest that fibrosis-related pathways could be targeted to prevent long-term liver damage in chronic HBV patients.

Future research should focus on functional validation of the identified genes and pathways using experimental models, such as CRISPR-based gene editing, siRNA knockdown and proteomic approaches. Moreover, integrating multi-omics data, including epigenomics, metabolomics and single-cell transcriptomics, could provide a more comprehensive understanding of HBV pathogenesis. By leveraging genomic insights and precision medicine approaches, targeted therapies can be developed to enhance immune responses, to prevent fibrosis and to improve clinical outcomes for HBV-infected patients.

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