



Comparative transcriptomic analysis of hepatocellular carcinoma according to primary liver disease using TCGA-LIHC data

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Abstract

Background and aim. Hepatocellular carcinoma arises in heterogeneous chronic liver conditions, including hepatitis B virus and hepatitis C virus infection, alcohol-associated liver disease, cirrhosis, and hepatic steatosis. The extent to which the tumor transcriptome reflects the underlying liver disease remains incompletely defined.

Methods. We analyzed publicly available data from The Cancer Genome Atlas Liver Hepatocellular Carcinoma cohort by clinical background, comparing hepatitis C virus-associated and hepatitis B virus-associated tumors with their respective adjacent non-tumor tissue, viral hepatitis-associated with alcohol-associated tumors, and tumors arising with viral hepatitis, alcohol use, or cirrhosis versus steatosis-associated tumors. Differential expression was assessed using an unpaired t-test with Benjamini-Hochberg correction and a fold-change threshold of 1.5.

Results. Hepatitis C virus-associated tumors showed 451 differentially expressed genes versus adjacent non-tumor tissue (61 upregulated, 390 downregulated); hepatitis B virus-associated tumors showed 500 (143 upregulated, 357 downregulated). Of these, 269 were shared and all were concordantly regulated, the most strongly downregulated corresponding to liver sinusoidal endothelial cell markers. Viral hepatitis-associated and alcohol-associated tumors differed by only three genes. Comparisons involving steatosis-associated tumors revealed broader differences (107 to 233 genes) dominated by cytochrome P450, drug, and retinol metabolism; however, that subgroup comprised only 12 tumors, and these findings are exploratory. Ten differentially expressed non-coding transcripts, comprising nine long non-coding ribonucleic acids and one pseudogene, were shared between the hepatitis B virus and hepatitis C virus comparisons. By contrast, the non-coding transcripts shared across the steatosis comparisons were sex-chromosome-linked, could not be distinguished from an effect of sex composition, and were not interpreted further.

Conclusion. Hepatitis B virus-associated and hepatitis C virus-associated tumors therefore share a substantial, concordant transcriptional program despite differing viral etiologies, whereas differences associated with the underlying liver disease are concentrated in hepatic metabolic and xenobiotic-processing pathways. These findings support the coexistence of a common transcriptional phenotype in hepatocellular carcinoma with etiology-associated molecular differences.

Keywords: carcinoma, hepatocellular, transcriptome, gene expression profiling, RNA, long noncoding, computational biology

Background and aims

Among liver cancers, hepatocellular carcinoma (HCC) is the most common primary liver cancer and remains a major cause of cancer-related mortality worldwide [1,2]. The development of HCC is strongly linked to chronic liver injury, with major risk factors including hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, alcohol-associated liver disease, and metabolic dysfunction-associated steatotic liver disease [1–3]. The epidemiological landscape of HCC is shifting as prevention and treatment of viral hepatitis improve, and the relative contribution of metabolic and alcohol-associated liver disease increases [2,4].

The mechanisms underlying malignant transformation vary with the underlying liver condition. HBV can contribute directly to carcinogenesis through viral deoxyribonucleic acid (DNA) integration and alterations in host-cell regulatory pathways, whereas HCV-associated carcinogenesis is largely linked to chronic inflammation, oxidative stress, fibrosis, and cirrhosis [1,5,6]. Alcohol-associated and steatotic liver disease also promote chronic inflammation and metabolic stress, but via partially distinct biological mechanisms [7]. Despite these differences, HCCs acquire common malignant traits; thus, etiologically distinct processes converge on shared molecular programs during tumor development [8,9].

Large-scale molecular profiling has revealed distinct genomic and transcriptomic heterogeneity in HCC. The Cancer Genome Atlas Liver Hepatocellular Carcinoma (TCGA-LIHC) project provided a comprehensive molecular characterization of HCC, identifying recurrent genomic alterations, molecular subtypes, and transcriptional programs with biological and clinical relevance [10]. Earlier transcriptome-based classifications had already shown that HCC comprises reproducible molecular subclasses: Boyault et al. defined six subgroups (G1–G6) related to clinical and genetic characteristics [11], and a meta-analysis by Hoshida et al. identified three subclasses reflecting WNT pathway activation (S1), proliferation with MYC and AKT activation (S2), and hepatocyte differentiation (S3) [12]. More recently, an analysis of the International Cancer Genome Consortium Japanese liver cancer cohort (LIRI-JP) showed that HBV- and HCV-associated HCC converge on similar tumor transcriptomic and immune profiles despite differences in the surrounding non-tumor liver tissue [13]; whether this convergence is also present in an ancestrally mixed cohort such as TCGA-LIHC has not been examined.

Long non-coding RNAs (lncRNAs) represent an additional layer of transcriptional regulation in HCC. Aberrant lncRNA expression has been implicated in tumor initiation, proliferation, invasion, and metastasis, and lncRNA profiles may vary by biological context [14]. Our study examined the lncRNA component as a secondary, exploratory layer within the broader transcriptomic analysis.

We aimed to compare gene expression patterns in the TCGA-LIHC cohort by underlying clinical background. Differentially expressed genes (DEGs) were examined in HBV- and HCV-associated tumors versus adjacent non-tumor tissue, and across tumors linked with viral hepatitis, alcohol use, cirrhosis, and hepatic steatosis. Shared and subgroup-associated transcriptional alterations were evaluated, with an exploratory assessment of differentially expressed lncRNA-annotated transcripts in HBV- and HCV-associated HCC.

Methods

TCGA-LIHC dataset and study groups

Publicly available data from the TCGA-LIHC cohort was accessed on 22 August 2026 and further bioinformatically analyzed. Samples were grouped by the clinical annotations used in the original analysis and by the presence of viral hepatitis, HBV infection, HCV infection, alcohol-associated liver disease, cirrhosis, or hepatic steatosis.

The overall dataset contained 243 HCC samples and 12 adjacent non-tumor samples. Etiology-specific tumor-versus-non-tumor analyses included 32 HCV-associated tumors and five HCV-associated adjacent non-tumor samples, and 75 HBV-associated tumors and seven HBV-associated adjacent non-tumor samples. Tumor-to-tumor comparisons included 114 viral hepatitis-associated HCC samples, 75 HBV-associated tumors, 32 HCV-associated tumors, 68 alcohol-associated tumors, 12 steatosis-associated tumors, and 231 tumors associated with cirrhosis. Because chronic liver conditions may coexist, these categories should not necessarily be interpreted as mutually exclusive.

Differential gene-expression analysis

We used normalized transcriptomic expression values for differential-expression analysis in Python. We performed comparisons using an unpaired t-test followed by Benjamini-Hochberg correction for multiple testing, with a corrected p-value < 0.05 considered statistically significant. A fold-change threshold of at least 1.5 was applied to both the HBV- and HCV-associated tumor-versus-adjacent non-tumor analyses and the comparisons between HCC subgroups by underlying liver disease. Genes meeting both the corrected significance threshold and the fold-change criterion were considered differentially expressed. Upregulation and downregulation refer to expression in the first group relative to the comparator group.

We performed a complementary, unstratified differential-expression analysis in GeneSpring GX version 15.5 (build 15.5.20939.0; Agilent Technologies). RNA-sequencing (RNA-seq) gene expression data were obtained from TCGA-LIHC through the UCSC Xena platform [15] (University of California, Santa Cruz), accessed on 22 August 2026, based on Illumina HiSeq 2000 RNA sequencing data generated by the University of North

Carolina. Gene expression profiles from 543 tumor tissue samples were compared with those from 13 normal liver tissue samples using an unpaired t-test with Benjamini–Hochberg correction; genes were considered differentially expressed when the absolute fold change exceeded 1.5, and the adjusted p-value was ≤ 0.05 . Genes with a fold change > 1.5 were classified as upregulated (408 genes), and genes with a fold change < -1.5 were classified as downregulated (848 genes). We generated volcano plots in GeneSpring to visualize the relationship between expression change magnitude and statistical significance, and heatmaps of the top 20 upregulated and top 20 downregulated genes to visualize expression patterns across tumor and normal tissue samples. We created Venn diagrams and bar graphs in Python.

Exploratory analysis of lncRNA-annotated transcripts

Transcripts annotated as long non-coding RNAs in the original dataset were extracted from the HBV and HCV tumor-versus-adjacent non-tumor differential expression results. We compared the two lncRNA lists to identify shared and virus-associated transcripts. Because the source dataset contains legacy transcript annotations, we mapped all transcript symbols to current Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC)-approved nomenclature before reporting [16]. Legacy identifiers are given in parentheses at first mention in the Results.

We derived DEGs lists for each pairwise contrast from TCGA-LIHC RNA-seq gene-expression data (UCSC Xena dataset TCGA.LIHC.sampleMap/HiSeqV2; Illumina HiSeq, $\log_2(\text{norm_count} + 1)$ values). Genes were considered differentially expressed at $|\log_2 \text{fold-change}| \geq 0.585$ and a Benjamini-Hochberg-adjusted $P < 0.05$ (t-test unpaired). We examined two complementary sets of contrasts. In the first, etiology-specific tumor signatures were each compared against a shared steatohepatitis tumor reference - cirrhotic (Cir-TT), alcoholic (Alc-TT), and viral (Vir-TT) - each versus steatosis-associated tumor tissue (Ste-TT). In the second, we compared tumor signatures

against adjacent normal tissue – HCV-associated tumor tissue (HC-TT) vs HCV-associated adjacent non-tumor tissue (HC-TN); HBV-associated tumor tissue (HB-TT) vs HBV-associated adjacent non-tumor tissue (HB-TN). We identified shared and set-specific genes by computing exact intersections of the DEG lists (set operations on official gene symbols) in Python 3.12.0. Overlaps were visualized as Venn diagrams with matplotlib v3.11.1 and matplotlib-venn v1.1.2; circles were drawn with a fixed, non-proportional layout so that all intersection compartments remained legible, and each compartment was annotated with its assigned gene symbols.

Pathway enrichment analysis

We performed pathway enrichment analysis using an over-representation approach based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) 2021 Human pathway collection [17,18]. We analyzed differentially expressed gene sets separately by direction of regulation and by clinical comparison. We paid particular attention to shared downregulated genes identified in both HBV- and HCV-associated HCC and to downregulated genes distinguishing steatosis-associated HCC from alcohol-, viral hepatitis-, HCV-, and HBV-associated tumors.

For each gene set, we evaluated overlap with individual KEGG pathways and calculated fold enrichment relative to expected pathway representation. We corrected for multiple testing using the Benjamini-Hochberg method and considered pathways with a false discovery rate (FDR) < 0.05 significantly enriched. We visualized enrichment results using bubble plots, where size represents the number of overlapping genes, and color indicates statistical significance as $-\log_{10}(\text{FDR})$.

The study used publicly available, de-identified TCGA data and did not require institutional ethics approval, as the Xena dataset identifier was already provided.

Results

Table I summarizes the transcriptomic comparisons performed in the TCGA-LIHC cohort and the number of differentially expressed genes identified in each analysis.

Table I. Summary of transcriptomic data from the TCGA-LIHC cohort.

Comparison	Group 1	Group 2	Upregulated	Downregulated	All DEGs
HCV tumor vs HCV non-tumor	32	5	61	390	451
HBV tumor vs HBV non-tumor	75	7	143	357	500
Viral hepatitis HCC vs alcohol HCC	114	68	0	3	3
HCV HCC vs alcohol HCC	32	68	2	10	12
HBV HCC vs alcohol HCC	75	68	3	17	20
Alcohol HCC vs steatosis HCC	68	12	33	74	107
Viral hepatitis HCC vs steatosis HCC	114	12	25	150	175
HCV HCC vs steatosis HCC	32	12	13	129	142
HBV HCC vs steatosis HCC	75	12	36	197	233
Cirrhosis-associated HCC vs steatosis HCC	231	12	24	123	147

Note: Upregulation and downregulation refer to expression in Group 1 relative to Group 2. DEG, differentially expressed gene; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus.

Differential expression in HCV- and HBV-associated hepatocellular carcinoma

Comparison of HCV-associated HCC with corresponding adjacent non-tumor liver tissue identified 451 differentially expressed genes (DEGs), of which 61 were upregulated, and 390 were downregulated in tumor tissue. Among the genes showing the largest decreases in HCV-associated HCC were C-type lectin domain family 4 member M (CLEC4M), C-type lectin domain family 1 member B (CLEC1B), growth differentiation factor 2 (GDF2), stabilin 2 (STAB2), secreted frizzled-related

protein 5 (SFRP5), C-type lectin domain family 4 member G (CLEC4G), and N-deacetylase and N-sulfotransferase 3 (NDST3).

The corresponding comparison between HBV-associated HCC and HBV-associated adjacent non-tumor tissue identified 500 DEGs, including 143 upregulated and 357 downregulated genes. Among the transcripts showing pronounced decreases were CLEC1B, CLEC4M, GDF2, synaptotagmin 9 (SYT9), CLEC4G, INS-IGF2 readthrough (INS-IGF2), NDST3, and STAB2.

Figure 1. Differential expression in HBV- and HCV-associated HCC

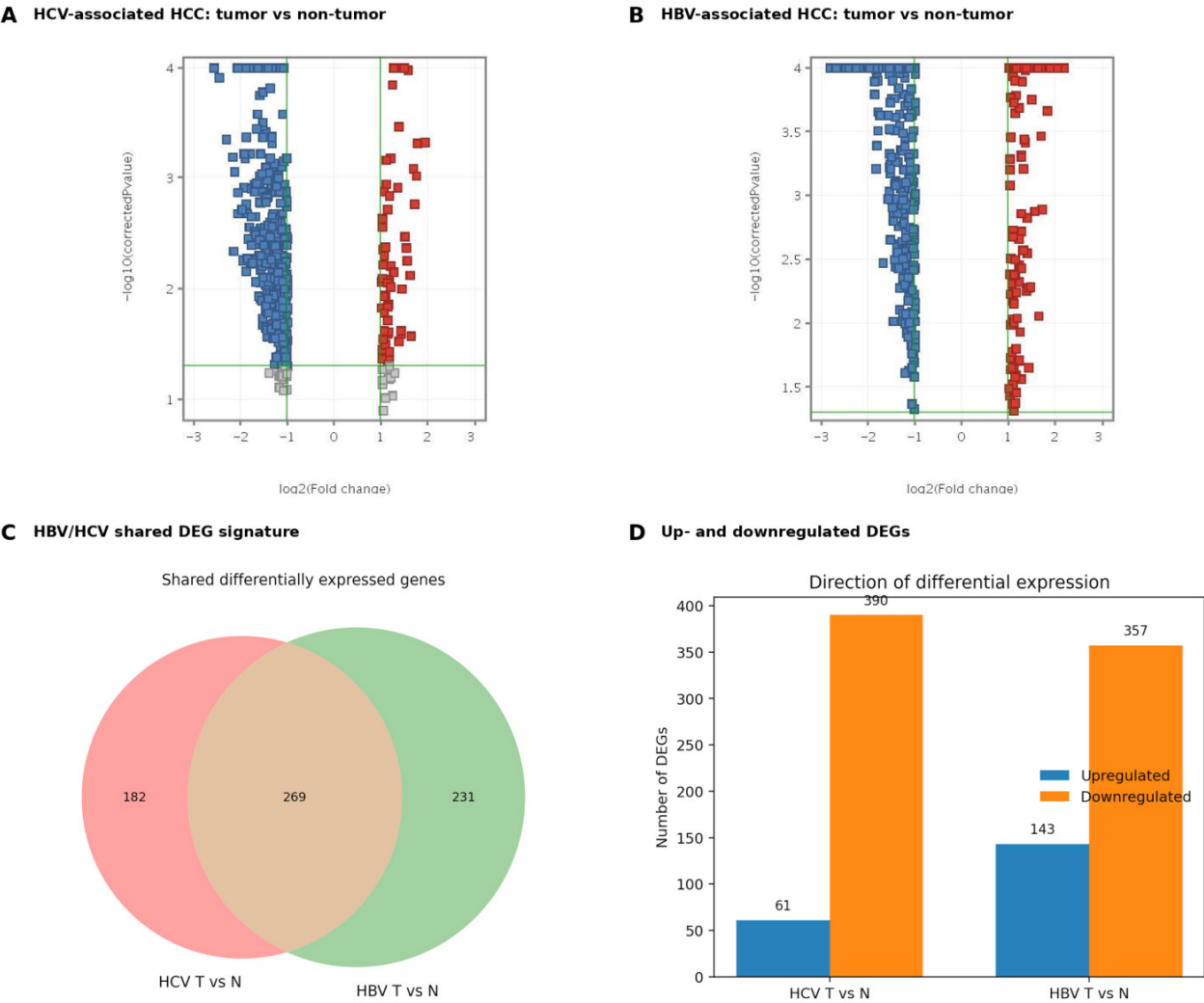


Figure 1. Differential expression in HBV- and HCV-associated HCC compared with adjacent non-tumor liver. (A) Volcano plot for HCV-associated HCC versus HCV-associated adjacent non-tumor tissue. (B) Volcano plot for HBV-associated HCC versus HBV-associated adjacent non-tumor tissue. (C) Overlap of the HCV and HBV DEG sets, showing 269 shared DEGs, 182 HCV-specific DEGs, and 231 HBV-specific DEGs. (D) Numbers of upregulated and downregulated DEGs in the two tumor-versus-non-tumor comparisons.

Comparison of the two DEG sets revealed substantial overlap between HBV- and HCV-associated HCC. A total of 269 genes were differentially expressed in both comparisons, representing 59.6% of the HCV-associated DEG set and 53.8% of the HBV-associated DEG set. An additional 182 DEGs were specific to the HCV-associated comparison, and 231 were specific to the HBV-associated comparison. Importantly, all 269 shared DEGs showed concordant direction of regulation in the two viral-associated HCC groups (Figure 1). The transcripts showing the largest decreases in both viral comparisons (CLEC4M, CLEC4G, CLEC1B, STAB2, and GDF2) are markers of liver sinusoidal endothelial cells and the hepatic sinusoidal niche and were concordantly downregulated in the HBV- and HCV-associated groups.

Limited transcriptomic differences between viral- and alcohol-associated HCC

A direct comparison of viral hepatitis-associated HCC and alcohol-associated HCC identified only three DEGs at the predefined significance and fold-change thresholds. These genes, NOVA1, GULP1, and MAGEA12, were expressed at lower levels in the viral hepatitis-associated group.

Relatively few differences were also observed when the viral groups were analyzed separately. Comparison of HCV-associated and alcohol-associated HCC identified 12 DEGs, including two upregulated and ten downregulated transcripts in the HCV group. Comparison of HBV-associated and alcohol-associated HCC identified 20 DEGs, of which three were upregulated and 17 were downregulated in the HBV group.

Figure 2. Transcriptomic differences between viral- and alcohol-associated HCC

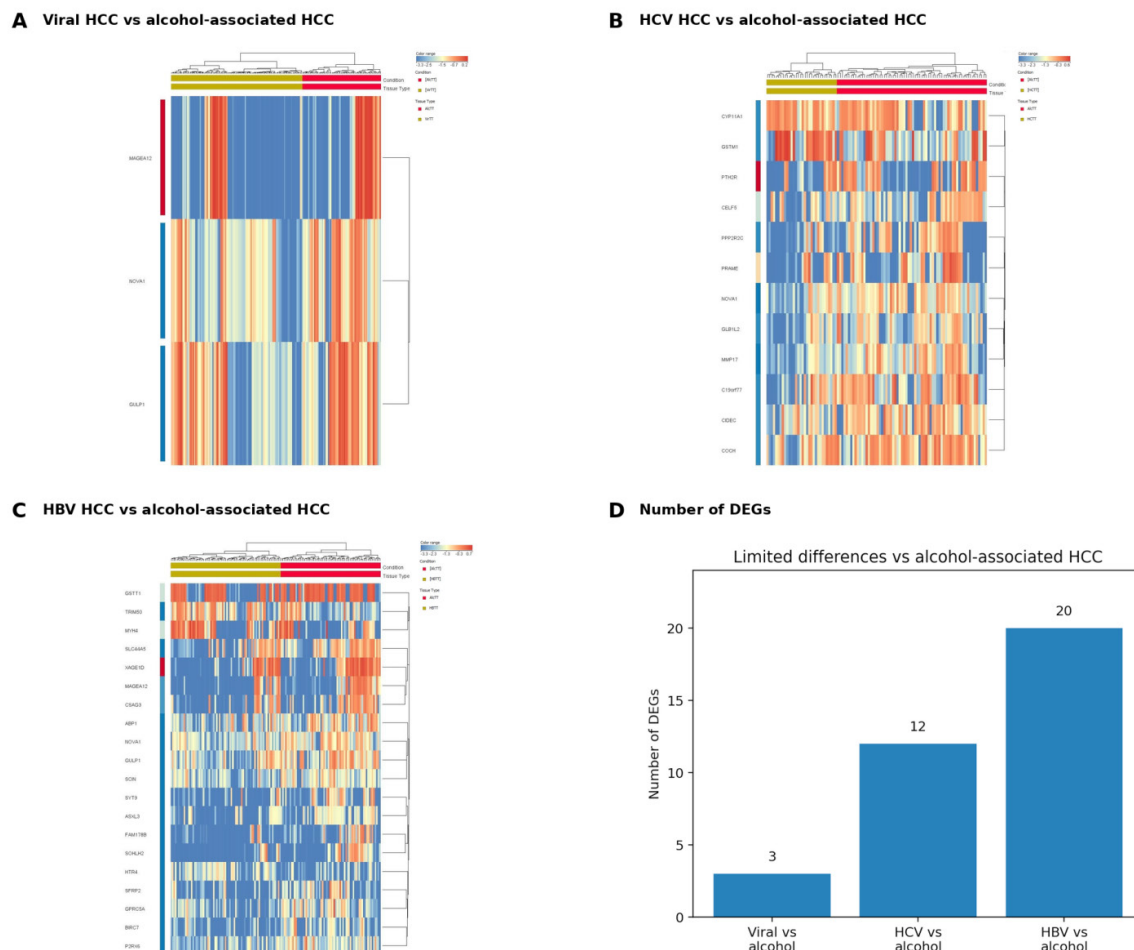


Figure 2. Transcriptomic differences between viral hepatitis- and alcohol-associated HCC.

Heatmaps show differentially expressed genes for (A) viral hepatitis-associated HCC versus alcohol-associated HCC, (B) HCV-associated HCC versus alcohol-associated HCC, and (C) HBV-associated HCC versus alcohol-associated HCC. (D) Bar chart summarizing the total number of differentially expressed genes (DEGs) identified in the three comparisons. For panels A–C, rows represent genes and columns represent individual samples, and the color scale reflects relative expression.

The limited number of significant transcripts in these comparisons suggests that the transcriptomic profiles of established viral- and alcohol-associated HCC are relatively similar under the analysis criteria used here (Figure 2).

Broader transcriptional differences involving steatosis-associated HCC

In contrast to the comparisons involving alcohol-associated tumors, substantially larger numbers of DEGs were identified when steatosis-associated HCC was used as the comparator. Comparison between alcohol-associated and steatosis-associated HCC identified 107 DEGs, including 33 genes expressed at higher levels and 74 expressed at lower levels in the alcohol-associated group.

The viral hepatitis-associated group differed from steatosis-associated HCC by 175 DEGs, of which 25 were

upregulated, and 150 were downregulated in viral hepatitis-associated tumors. When HCV-associated tumors were analyzed separately, 142 DEGs were detected compared with steatosis-associated HCC, with 13 genes upregulated and 129 downregulated in the HCV-associated group.

The largest number of differences was detected between HBV-associated and steatosis-associated HCC, with 233 DEGs, including 36 upregulated and 197 downregulated genes. Comparison between cirrhosis-associated and steatosis-associated tumors identified 147 DEGs, including 24 upregulated and 123 downregulated genes (Table I). Overall, the larger number of differentially expressed transcripts in comparisons involving the steatosis-associated subgroup suggests a more distinct transcriptional profile in this subgroup within the present cohort (Figure 3).

Figure 3. Steatosis-associated HCC shows broader transcriptomic differences

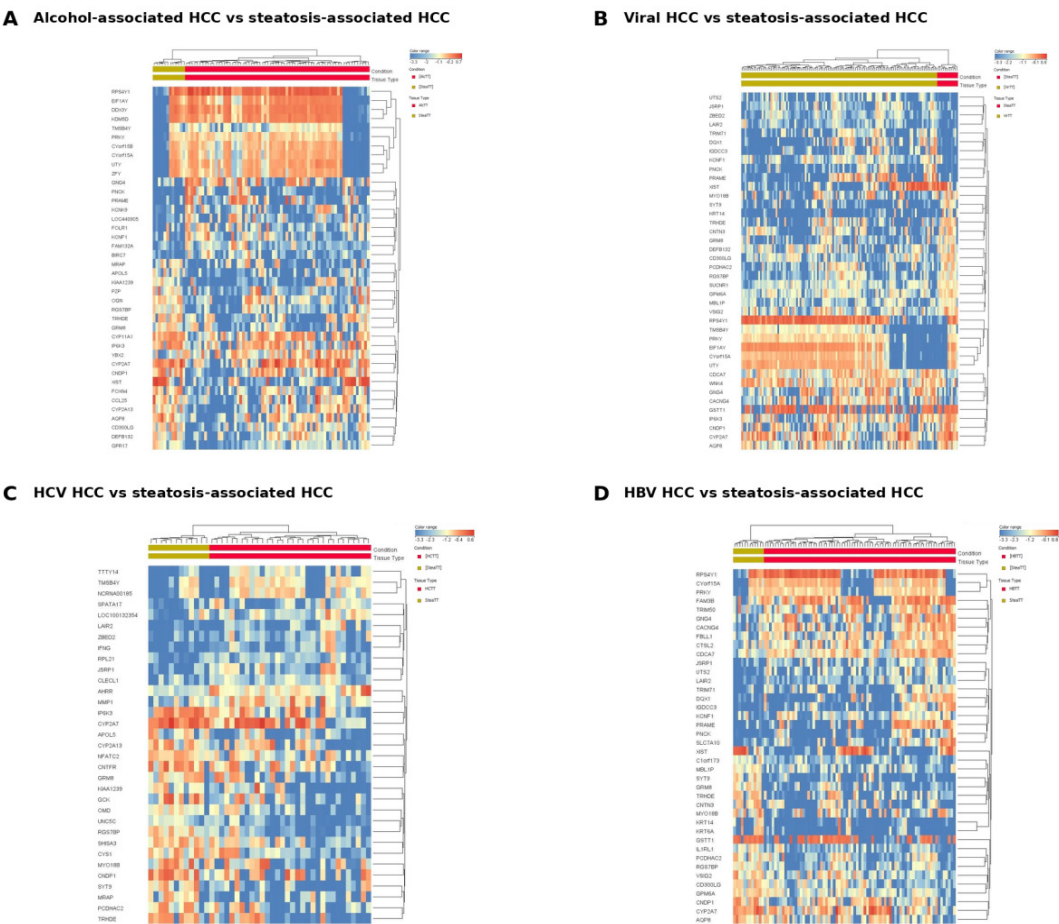


Figure 3. Transcriptomic differences between steatosis-associated HCC and other HCC subgroups. Heatmaps show differentially expressed genes for (A) alcohol-associated HCC versus steatosis-associated HCC, (B) viral hepatitis-associated HCC versus steatosis-associated HCC, (C) HCV-associated HCC versus steatosis-associated HCC, and (D) HBV-associated HCC versus steatosis-associated HCC. Rows represent genes and columns represent individual samples; the heatmap color scale reflects relative expression values.

Shared and distinct lncRNA alterations in HBV- and HCV-associated HCC

Analysis of transcripts annotated as long non-coding RNAs identified 15 differentially expressed non-coding transcripts in HCV-associated HCC compared with adjacent non-tumor tissue, and 18 in the corresponding HBV-associated comparison. Ten transcripts were common to both analyses (Figure 4A). Because the source dataset carries a legacy annotation, all symbols are reported below in parentheses: LINC01550 (C14orf64), LINC02875 (C17orf82), B3GALT5-AS1 (C21orf88), CDKN2B-AS1 (CDKN2BAS), FAM99B, LINC01512 (LOC100132354), LY6E-DT (LOC100133669), HAND2-AS1 (NBLA00301), UCA1, and FAM169BP (FAM169B). Of these ten, nine are annotated as long non-coding RNAs in current references; FAM169BP is annotated as a pseudogene and is therefore reported separately and excluded from lncRNA-specific

statements. Five transcripts were detected only in the HCV comparison — ALMS1P1 (ALMS1P), LY6S-AS1 (C8orf31), DOCK8-AS1 (C9orf66), FAM30A (KIAA0125) and LINC00092 (NCRNA00092) – and eight only in the HBV comparison: TSPEAR-AS2 (C21orf90), LINC01554 (C5orf27), ZNF252P-AS1 (C8orf77), FAM99A, INE2, LINC00685 (NCRNA00107), TTTY14 (NCRNA00185) and SFTA1P.

The shared component shows that HBV- and HCV-related HCC share a core set of non-coding transcriptional changes, despite their different viral causes. This aligns with the large overlap seen among protein-coding DEGs. Transcripts found only in one viral subgroup imply that a smaller portion of non-coding variation might be linked to the specific viral context. However, with only 5 and 8 subgroup-specific transcripts, respectively, these differences are minor and may reflect detection limits more than biological factors.

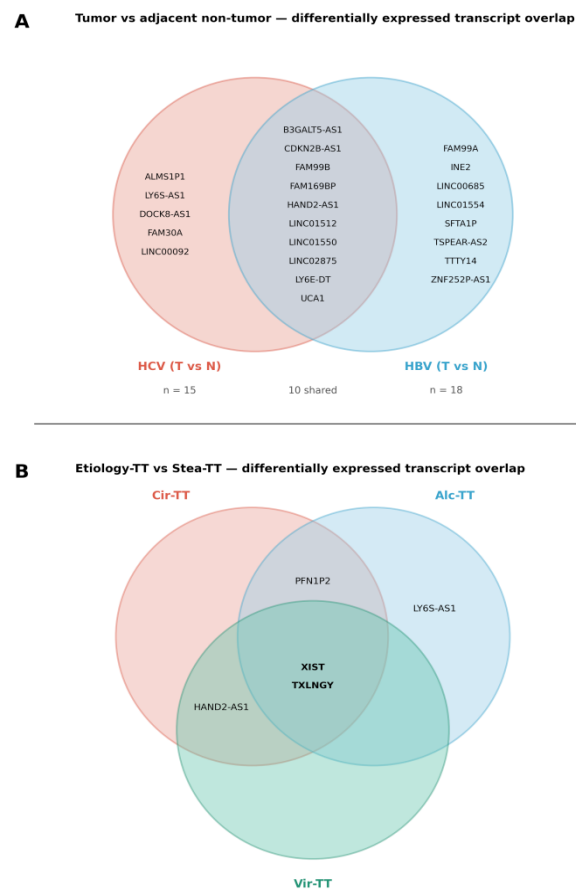


Figure 4. Overlap of differentially expressed lncRNA-annotated transcripts across HCC comparisons in TCGA-LIHC.

(A) Venn diagram showing the overlap of lncRNA-annotated transcripts differentially expressed in tumor versus adjacent non-tumor tissue for HCV-associated HCC (HC, T vs N; red) and HBV-associated HCC (HB, T vs N; blue). Fifteen transcripts were identified in the HCV comparison and 18 in the HBV comparison, of which 10 were shared. (B) Venn diagram showing the overlap of lncRNA-annotated transcripts differentially expressed between steatosis-associated tumor tissue (Stea-TT) and, respectively, cirrhosis-associated (Cir-TT; red), alcohol-associated (Alc-TT; blue) and viral hepatitis-associated (Vir-TT; teal) tumor tissue.

Two caveats apply to this layer. First, the set is annotationally heterogeneous. Of the ten shared transcripts, nine are current lncRNAs and one (FAM169BP) is a pseudogene; among the subgroup-restricted transcripts, ALMS1P1 is a pseudogene, and SFTA1P is a pseudogene-derived lncRNA. The group is therefore more accurately described as non-coding-annotated transcripts than as lncRNAs, and pseudogene entries are kept separate from lncRNA statistics throughout. Second, the analysis used a legacy TCGA annotation in which many transcripts carried locus-based identifiers that have since been reassigned. All symbols were mapped to current HGNC nomenclature before reporting, and the corresponding legacy identifier is given in parentheses at first mention. For the HBV-only set, warrant particular note – TTTY14 is Y-linked [19] and its presence in a comparison of 75 tumors against 7 adjacent non-tumoral tissue samples may in fact reflect the sex-composition imbalance between the two groups rather than the actual tumor biology.

To determine whether non-coding alterations also distinguish HCC subgroups defined by underlying liver disease, we compared non-coding transcripts across tumor tissues associated with cirrhosis, alcohol, and viral hepatitis, and with steatosis (Figure 4B). The three comparisons yielded few differentially expressed transcripts and limited overlap. Two entries, XIST and TXLNGY (CYorf15A/CYorf15B), were common to all three comparisons; PFN1P2 (C1orf152) was shared between the cirrhosis and alcohol comparisons; HAND2-AS1 (NBLA00301) was shared between the cirrhosis and viral comparisons; and LY6S-AS1 (C8orf31) was restricted to the alcohol comparison. We note that CYorf15A and CYorf15B are previous symbols for a single locus, TXLNGY, and represent one gene rather than two.

This result is not interpretable as an etiology-associated finding. XIST is the master regulator of X-chromosome inactivation and is expressed almost exclusively in females, whereas TXLNGY is a Y-linked pseudogene expressed only in males; the two transcripts shared across all three comparisons are therefore sex-chromosome-linked, and a third shared entry, PFN1P2, is a pseudogene. Sex was not included as a variable in the differential-expression model, and with only 12 tumors in the steatosis-associated group, a difference in sex ratio between that group and its comparators cannot be excluded as an explanation for these transcripts. Because an etiology-associated interpretation cannot be distinguished from a sex-composition effect using the present analysis, we do not interpret this comparison further, and the non-coding findings of this study are restricted to the shared HBV/HCV component reported above.

Transcript symbols are shown within the corresponding regions; regions without labels contained no transcripts unique to that intersection. Transcripts were considered differentially expressed at a Benjamini-

Hochberg-adjusted $p < 0.05$ with an absolute fold change ≥ 1.5 . Transcript symbols are shown in current HGNC nomenclature; legacy identifiers from the source annotation are given in parentheses at first mention in the Results. Several entries are annotated as pseudogenes rather than long non-coding RNAs. Note that the transcripts shared across all three comparisons in panel B (XIST, TXLNGY) are sex-chromosome-linked.

KEGG pathway enrichment analysis

We performed KEGG pathway enrichment analysis to explore the biological processes associated with the identified differential-expression signatures [17,18]. Of the 269 DEGs shared between the HBV- and HCV-associated comparisons, 230 were downregulated, and 39 were upregulated in tumor tissue. Among the 230 commonly downregulated genes, eight KEGG pathways were statistically significant after multiple-testing correction. The strongest enrichment was observed for cytokine–cytokine receptor interaction ($FDR = 4.74 \times 10^{-4}$), followed by ECM–receptor interaction ($FDR = 0.0196$), viral protein interaction with cytokine and cytokine receptor ($FDR = 0.0248$), cell adhesion molecules ($FDR = 0.0248$), and neuroactive ligand–receptor interaction ($FDR = 0.0248$). Significant enrichment was also observed for the transforming growth factor beta (TGF- β) signaling pathway, axon guidance, and mineral absorption ($FDR = 0.0425$ for each) (Figure 5A). In contrast, no KEGG pathway reached an $FDR < 0.05$ among the 39 shared upregulated genes.

We also examined pathway enrichment for the downregulated gene sets identified in comparisons involving steatosis-associated HCC. In the HBV-associated HCC versus steatosis-associated HCC comparison, significant enrichment was observed for metabolism of xenobiotics by cytochrome P450 ($FDR = 0.0028$), drug metabolism ($FDR = 0.0098$), and retinol metabolism ($FDR = 0.0416$). Similarly, the combined viral hepatitis-associated HCC versus steatosis-associated HCC comparison showed enrichment of xenobiotic metabolism ($FDR = 0.0118$), drug metabolism ($FDR = 0.0200$), retinol metabolism ($FDR = 0.0208$), and axon guidance ($FDR = 0.0216$).

For HCV-associated HCC versus steatosis-associated HCC, the strongest enrichment was detected for axon guidance ($FDR = 0.0047$), together with maturity-onset diabetes of the young ($FDR = 0.0114$). In the alcohol-associated HCC versus steatosis-associated HCC comparison, significant enrichment included metabolism of xenobiotics by cytochrome P450 ($FDR = 0.0301$) and drug metabolism ($FDR = 0.0367$), among other pathways. Overall, these findings indicate that the common HBV/HCV transcriptional signature is associated predominantly with cytokine signaling, extracellular-matrix interactions, cell adhesion, and related signaling processes, whereas differences involving steatosis-associated HCC are characterized mainly by alterations in metabolic and xenobiotic-processing pathways (Figure 5B).

KEGG pathway enrichment of TCGA-LIHC DEG signatures

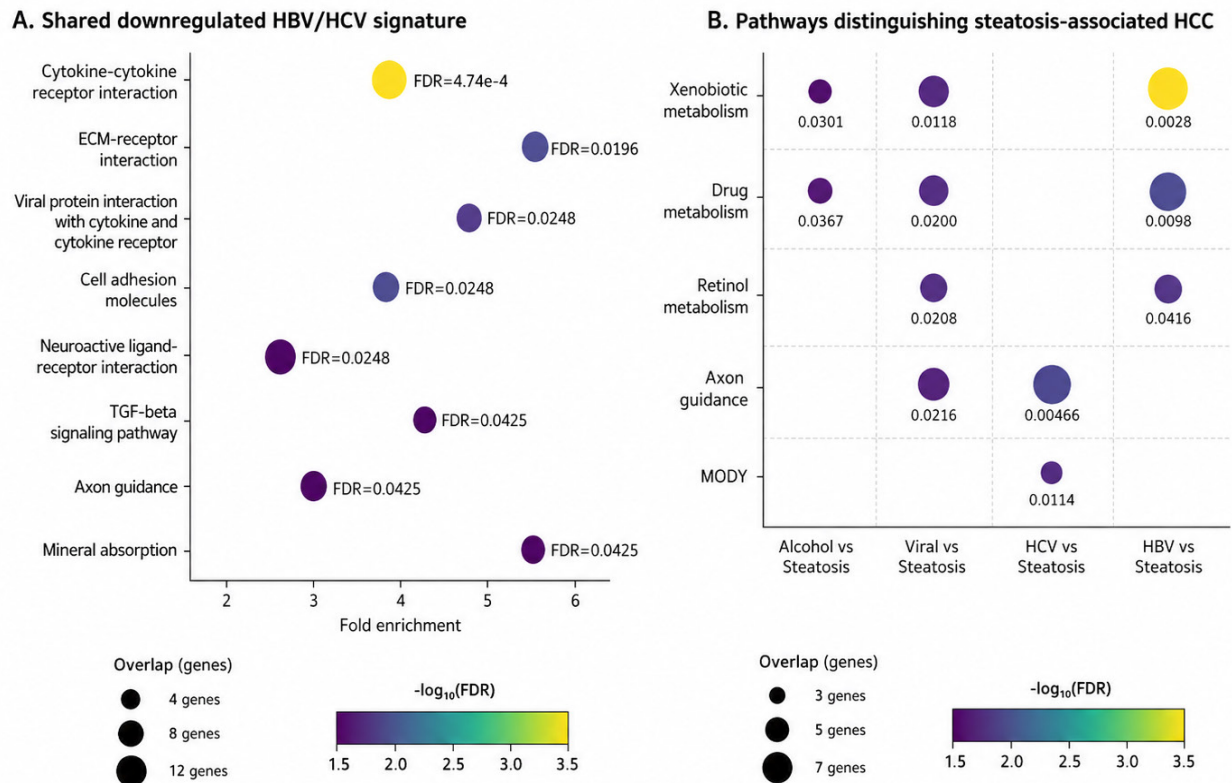


Figure 5. KEGG pathway enrichment analysis of TCGA-LIHC DEG signatures.

(A) Selected pathways enriched among genes commonly downregulated in HBV- and HCV-associated HCC. (B) Selected pathways enriched in comparisons involving steatosis-associated HCC. Bubble size indicates the number of overlapping genes, and color represents $-\log_{10}(\text{FDR})$. Only pathways with $\text{FDR} < 0.05$ are shown.

Discussion

The present analysis demonstrates that the transcriptomic landscape of HCC contains both a substantial common tumor-associated component and differences related to the underlying liver disease. The clearest evidence of molecular convergence was observed in HBV- and HCV-associated HCC. Although HBV and HCV differ in viral biology and mechanisms of hepatocarcinogenesis, 269 DEGs were shared between their respective tumor-versus-adjacent non-tumor comparisons, and every shared transcript showed concordant regulation.

This observation is consistent with the concept that distinct causes of chronic liver injury can converge on common molecular programs during malignant transformation. A recent transcriptomic analysis of HBV- and HCV-mediated HCC in an independent Japanese cohort similarly reported convergence toward related tumor transcriptomic landscapes despite greater differences in the surrounding non-tumor tissue [13]; the present results

extend this observation to TCGA-LIHC. The present results therefore support a shared viral hepatitis-associated HCC transcriptional program while also identifying substantial numbers of HBV- and HCV-specific DEGs. Because established molecular subclasses of HCC are related to clinical and genetic characteristics [11,12], this shared program should be interpreted as convergence in tumor-versus-adjacent-tissue change rather than as evidence that HBV- and HCV-associated tumors belong to a single molecular class; tumors were not assigned to the G1–G6 or S1–S3 subclasses in this analysis.

Several of the genes showing the most pronounced decreases in both viral-associated HCC groups are markers of the hepatic sinusoidal niche. CLEC4M, CLEC4G, CLEC1B, STAB2 and GDF2 were consistently reduced in HBV- and HCV-associated tumors. Single-cell profiling of human liver has established that CLEC4G, CLEC4M and STAB2 are expressed predominantly by liver sinusoidal endothelial cells (LSECs) [20], while GDF2, which encodes

BMP9, is a paracrine factor that maintains LSEC fenestration and protects against hepatic fibrosis [21]. Their coordinated reduction is consistent with sinusoidal capillarization, the transdifferentiation of fenestrated sinusoidal endothelium toward a continuous phenotype, an established feature of chronic liver disease and HCC [22]. Loss of stabilin-2 expression has been demonstrated immunohistochemically in HCC nodules and in peritumoral liver, where it correlates with survival [23], providing histological support for the transcriptional signal observed here. However, bulk RNA sequencing measures a mixture of cell types, and part of this reduction may reflect a lower proportion of sinusoidal endothelial cells in tumor tissue relative to adjacent liver rather than loss of endothelial identity within those cells. We did not estimate tumor purity, for example with ESTIMATE [24] or ABSOLUTE [25], or deconvolve cell-type proportions, for example with CIBERSORTx [26], so these explanations cannot be separated in the present analysis. The recurrence of this pattern across both viral backgrounds indicates that loss of specialized sinusoidal identity is a component of the shared viral hepatitis-associated HCC phenotype rather than an etiology-specific finding. Among the additional altered genes, SFRP5 has been reported to undergo epigenetic silencing in HCC with enhanced Wnt signaling [27] whereas NDST3, SYT9 and the INS-IGF2 readthrough transcript have no established primary literature in liver tissue and are reported here as hypothesis-generating observations requiring independent confirmation.

The observed convergence was not restricted to the protein-coding transcriptome. Fifteen differentially expressed non-coding transcripts were identified in HCV-associated HCC and 18 in HBV-associated HCC, with ten shared between the two tumor-versus-adjacent non-tumor comparisons: LINC01550, LINC02875, B3GALT5-AS1, CDKN2B-AS1, FAM99B, LINC01512, LY6E-DT, HAND2-AS1, UCA1 and the pseudogene FAM169BP. Nine of these ten are annotated as lncRNAs in current references, and the presence of a shared non-coding component suggests that molecular convergence between HBV- and HCV-associated HCC extends beyond protein-coding genes [14]. Four of the shared transcripts have been functionally characterized in HCC. CDKN2B-AS1 (ANRIL) promotes tumor growth and metastasis through a let-7c-5p/NAP1L1 axis [28]; UCA1 is upregulated by HBx and promotes cell growth through EZH2 recruitment and p27 repression [29]; FAM99B is a liver-specific lncRNA that suppresses proliferation, migration and invasion [30], as is its neighbor FAM99A [31]; and B3GALT5-AS1 acts as a suppressor via a miR-934/UFM1 axis [32]. The evidence for HAND2-AS1 is directionally contradictory, with reports of both tumor-suppressive activity through a miR-300/SOCS5 axis [33] and oncogenic activity promoting liver cancer stem cell self-renewal via BMP signaling [34]; we report this conflict rather than favoring

either direction. LINC01512 has been characterized in lung adenocarcinoma, but not in HCC [35], and LINC01550, LINC02875 and LY6E-DT have, to our knowledge, no primary functional characterization in any cancer. Because this analysis was exploratory, the number of adjacent non-tumor samples was limited, and several transcripts remain uncharacterized; these observations require validation in independent cohorts.

By contrast, the non-coding comparison between steatosis-associated tumors and the other etiological subgroups did not yield an interpretable result. The transcripts shared across all three comparisons were XIST and TXLNGY, both sex-chromosome-linked, together with the pseudogene PFN1P2. Because sex was not modeled and the steatosis-associated group was small, we cannot attribute these transcripts to etiology rather than group composition, and we therefore draw no conclusion from this comparison. This asymmetry illustrates a broader methodological point: in small, clinically defined subgroups, demographic composition can influence differential-expression results, and sex-chromosome transcripts provide a readily recognizable indicator that this may have occurred.

Functional enrichment analysis further supported the biological relevance of the shared HBV/HCV transcriptional signature. The commonly downregulated genes were significantly enriched in pathways related to cytokine–cytokine receptor interactions, extracellular matrix–receptor interactions, cell adhesion molecules, and TGF- β signaling. These processes are closely linked to hepatic microenvironment organization, intercellular communication, immune regulation, and tissue remodeling. Their coordinated alteration in both HBV- and HCV-associated HCC therefore suggests that the transcriptional convergence observed between the two viral backgrounds reflects common changes in tumor–microenvironment interactions and signaling networks, rather than overlap only at the level of individual genes.

The limited number of DEGs observed when viral hepatitis-associated tumors were compared directly with alcohol-associated HCC provides a complementary observation. Only three genes met the predefined criteria in the viral-versus-alcohol comparison, while 12 and 20 DEGs were identified in the HCV-versus-alcohol and HBV-versus-alcohol comparisons, respectively. These findings suggest that, within the present dataset and under the selected thresholds, established HCCs associated with viral hepatitis and alcohol use show relatively limited transcriptomic separation.

A different pattern emerged in comparisons involving steatosis-associated HCC. Between 107 and 233 DEGs were identified when steatosis-associated tumors were compared with alcohol-, viral hepatitis-, HCV, or HBV-associated HCC, and 147 DEGs were detected in the cirrhosis-versus-steatosis comparison. This observation is of interest in the context of the changing epidemiology

of HCC, in which metabolic and steatotic liver disease has become increasingly important [2]. HCC arising in a steatotic or metabolic background develops within a biological environment characterized by altered lipid metabolism, insulin resistance, inflammatory signaling, and metabolic stress [36–38]. Steatosis in the background liver should also be distinguished from steatohepatic HCC (SH-HCC), a histological variant showing large-droplet steatosis, ballooning of malignant hepatocytes, Mallory-Denk bodies, inflammation, and pericellular fibrosis [39] and frequent interleukin-6 (IL-6)/Janus kinase (JAK)/signal transducer and activator of transcription (STAT) activation without CTNNB1, TERT, or TP53 alterations [40]. The clinical annotation used to define the steatosis-associated group describes the background liver rather than tumor histology, so these 12 tumors cannot be assumed to represent SH-HCC. Nevertheless, the steatosis-associated group in the present analysis contained only 12 tumors, so the observed differences should be interpreted as exploratory, etiology-associated differences requiring independent validation.

The pathway analysis also provided functional support for the greater transcriptomic divergence observed in steatosis-associated HCC. Comparisons of viral-, HBV-, and alcohol-associated tumors with steatosis-associated HCC consistently identified enrichment of pathways related to xenobiotic metabolism, drug metabolism, and retinol metabolism, largely involving cytochrome P450-associated genes. These findings suggest that differences in hepatic metabolic and detoxification programs contribute to the distinct transcriptomic profile of steatosis-associated tumors. Altered metabolic function is a recognized feature of steatotic liver disease and may influence both hepatocyte biology and the microenvironment in which malignant transformation occurs. Nevertheless, given the relatively small number of steatosis-associated tumors in the present cohort, these pathway-level differences should be considered exploratory and require confirmation in independent datasets. Downregulation of xenobiotic-metabolizing cytochromes P450 relative to surrounding tissue is a documented feature of HCC [41], and altered hepatic metabolic and detoxification capacity is characteristic of steatotic liver disease [36–38]. It should be noted, however, that histologically non-tumoral liver adjacent to HCC carries pre-malignant transcriptional changes [12], so metabolic differences observed between tumor subgroups may partly reflect the metabolic state of the liver in which the tumor arose rather than a tumor-intrinsic property.

Several limitations should be considered. First, this is a retrospective analysis of a publicly available dataset, and the clinical groups were unequal in size; the steatosis-associated subgroup ($n = 12$) and the adjacent non-tumor groups ($n = 5$ for HCV and $n = 7$ for HBV) were particularly small. Differential-expression analyses

based on very few replicates are known to show reduced reproducibility and inflated false-positive rates [42,43], and the subgroup comparisons reported here should therefore be regarded as hypothesis-generating. Second, we assessed differential expression using an unpaired parametric t-test on normalized expression values rather than a count-based model designed for RNA-sequencing data, which is less well calibrated at small and unbalanced group sizes [42]. Third, the etiological strata are not distributed evenly with respect to genetic ancestry: HBV-associated cases are enriched for East Asian ancestry, whereas HCV- and alcohol-associated cases are enriched for European ancestry. Because ancestry is associated with molecular differences across tumor types [44], the HBV-versus-HCV and HBV-versus-alcohol contrasts are partially confounded by ancestry and cannot be interpreted as isolating viral etiology. Fourth, we did not include sex as a variable in the differential-expression model. The appearance of sex-chromosome transcripts among the steatosis comparisons indicates that group composition may have influenced those results, which were consequently excluded from interpretation; comparisons in small clinical subgroups would benefit from adjustment for sex in future work. In addition, we did not adjust for tumor purity or cell-type composition, which may contribute to differences involving stromal and endothelial transcripts [24,26]. Fifth, underlying liver conditions may overlap, and the clinical categories should not be interpreted as completely independent etiologies; the cirrhosis stratum in particular overlaps extensively with the viral and alcohol strata. Sixth, the source dataset carries a legacy transcript annotation, and although all symbols were mapped to current HGNC nomenclature, several transcripts remain functionally uncharacterized. Finally, the study is descriptive and does not include independent external validation or functional experiments.

Conclusions

Our transcriptomic analysis of the TCGA-LIHC cohort revealed a substantial shared gene-expression program between HBV- and HCV-associated HCC, with 269 common DEGs showing concordant regulation. The most strongly downregulated shared transcripts were markers of liver sinusoidal endothelial cells, indicating that loss of specialized sinusoidal identity forms part of the shared viral hepatitis-associated phenotype. Direct differences between viral hepatitis- and alcohol-associated HCC were relatively limited, whereas comparisons involving steatosis-associated tumors identified considerably larger numbers of differentially expressed transcripts, dominated by hepatic metabolic and xenobiotic-processing pathways; given the small size of this subgroup ($n = 12$), these differences should be regarded as exploratory. HBV- and HCV-associated HCC also shared ten differentially expressed non-coding transcripts, comprising nine long non-coding RNAs and one pseudogene. Together, these findings illustrate the

coexistence of a common HCC transcriptional phenotype and molecular differences associated with the underlying liver disease.

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Authors' contribution

A.M.C. contributed to writing the original draft, clinical interpretation of the data, and critical revision of the manuscript. **P.C.** contributed to methodology, bioinformatic analysis, writing of the original draft, and critical revision of the manuscript. **C.C.** contributed to bioinformatic analysis, writing of the original draft, and critical revision of the manuscript. **A.T.** and **D.G.C.** contributed to interpretation of the data and writing of the original draft. **I.R.** contributed to clinical interpretation of the data and critical revision of the manuscript. **S.S.** contributed to statistical methodology, interpretation of the data, and critical revision of the manuscript. **I.B.-N.** contributed to methodology, bioinformatic analysis, project administration, writing of the original draft, and critical revision of the manuscript. **N.A.H.** contributed to supervision of the study. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work

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