Hepatitis B Virus Associated Hepatocarcinoma Activated Unique Metabolism Pathway to Gain Survival Advantages

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Research

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Abstract

Background: Hepatitis B Virus (HBV) is considered as a “metabolic virus” and affects the hepatic metabolism homeostasis, while except for the anti-virus therapy the principle of HBV-negative hepatocellular carcinoma (HCC) and HBV-positive HCC is exactly the same until now. Exploration of the unique characteristics of HBV-associated HCC will provide new metabolic targets against HBV-associated HCC to gain survival advantage, and finally inhibit cancer progression.

Methods: To systematically assess the metabolic changes, we assessed the transcriptomic and proteomic data from public database, additionally verified the accordingly changes in our own test cohort on proteome and transcriptome level. Additionally, the tissue metabolomics profile, lipidomics and the activity of PI3K/AKT/mTOR signaling pathway which play critical role in metabolism reprogramming, were analyzed to confirm the aforementioned changes.

Results: By multi-omics approaches, we presented the metabolic dysfunction experienced during HBV-associated HCC, represented by upregulated the steroid hormone biosynthesis, primary bile acid, and sphingolipid metabolism which related to a poor prognosis. Metabolites in linoleic acid metabolism and primary bile acid metabolism were closely correlated with HBV replication in HBV-positive HCC patients. Further tissue metabolomics and lipidomics validate the serum metabolic changes, namely changing in lipid composition and unsaturated fatty acid accumulation. By antibody array, a hyperactive PI3K/AKT/mTOR signaling transduction in HBV-positive biopsy compared with HBV-negative patients was observed, indicating a key role of this pathway in metabolic reprogramming of HBV-associated HCC.

Conclusion: HBV plays a critical role in the metabolism of HBV-associated HCC and activated a unique metabolism pathway to gain survival advantages. HBV-positive HCC showed obviously difference compared to HBV-negative HCC in metabolism at least and should be treated according to its characteristics in the clinic.

Background

As one of the most malignant cancers worldwide, hepatocellular carcinoma ranked as the fourth leading cause of cancer-related death, with an estimated incidence of more than 1 million by 2025 [1]. Recent advances in early diagnosis and treatment have improved the short-term prognosis of patients with HCC, but the long-term prognosis remains poor even after curative treatment [2, 3]. HBV is the predominant risk factor for developing HCC, which accounts for 85% HCC cases in Asia. HBV induced metabolic dysregulation in hepatitis has been investigated for many years, while the role of HBV in HCC metabolism remains unclear. It becomes more and more widely accepted that oncogenic DNA virus could hijack the cell metabolic machinery caused metabolic rearrangement sequentially [4].

Moreover, aside from the anti-virus treatment for HBV-associated HCC, the recommended interventions for HBV-negative HCC and HBV-positive HCC is exactly the same. Exploration of the unique characteristics of HBV-associated HCC will provide new metabolic targets against HBV-associated HCC to gain survival

advantage, and finally inhibit cancer progression. HBV is considered a “metabolic virus” and affects many hepatic metabolic pathways, especially the lipid metabolism including fatty acid metabolism, phospholipids and cholesterol [5]. The large-sample prospective cohort study showed that HBsAg positivity was inversely correlated with fatty liver [6]. For example, HBsAg seropositivity was associated with a lower risk of developing nonalcoholic fatty liver disease during an approximately 10-year follow-up of 83,339 non-NAFLD Korean adults with normal alanine aminotransferase levels at baseline [7].

The studies on the metabolism characteristics of HBV-associated HCC are limited [8]. The most well-studied metabolic change is the Warburg effect and glutamine metabolism [6, 9]. Metabolites influenced by metabolic reprogramming also exert cancer-promoting functions by modulating signaling pathways, epigenetic states, and cellular differentiation. Lipid metabolic reprogramming in carcinogenesis has been considered as an alternative treatment target in HCC [10]. In a preliminary cell experiments, total fatty acids were found increased in HepG2 cells [11]. Studies using an HBV infection cell model showed that lipid metabolites such as N-acetyl aspartate and choline were reduced in HepG2 cells [12]. Using an *in vitro* HBV infection model, researchers identified metabolic signatures, the hexosamine and the phosphatidylcholine biosynthesis disruption, was associated with HBV replication [13]. Therefore, a multi-omics study based on patients is needed to clarify the landscape of metabolic dysfunction of HBV associated HCC.

In this study, a multi-omics study involved with 318 HCC patients were performed based on transcriptome, proteomics and metabolomics technique to investigate the implication of HBV on the metabolism of HCC. Firstly, the transcriptional and proteomic characteristics of 224 HCC tissue of patients were analyzed by using public database included The Cancer Genome Atlas program (TCGA) and Chinese Human Proteome Project (CNHPP) liver data portal. 92 HCC patients’ serum protein signature by protein array, as well as the plasma metabolites profile by ultra-performance liquid chromatography coupled with electrospray ionization/quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q-TOF-MS) to summarize the metabolites profile in patients as well as to confirm the results from public database. Furtherly, the biopsies collected from clinical HBV-positive/negative HCC patients was analyzed to verify the metabolic and lipidomic profile. Eventually, the related metabolic signaling cascade was probed in situ. This study indicated that HBV-associated HCC showed unique metabolism processes to gain survival advantage and should be treated individually, which also provides potential targets for treatments.

Materials And Methods

Patient recruitment and sample collection

Ninety-two participants who administered in the department of integrative medicine in Fudan affiliated cancer center from 1 January 2016 and 31 May 2017 were enrolled in this study, 28 health controls, 49 HBV-positive HCC, and 15 HBV-negative HCC, characteristics of the patients were listed in Table. 1. To avoid the gender difference on metabolites, only male was enrolled in this study. Patients’ information,

serum, plasma, and biospecimens of patients were collected from the minimally invasive therapy center in Fudan University Shanghai Cancer Center with informed consent as per the approval of the IRB (No. 1904200-13). Blood samples were collected in anti-coagulation tubes containing potassium-EDTA (Becton Dickinson, Franklin Lakes, NJ, USA), which transported to the laboratory for processing. Serum was prepared by collecting blood in a serum separator tube by centrifuging (1000 g) at 4 degree for 10 minutes after clotting. Plasma was separated by collecting blood into purple top EDTA tubes and centrifuged (2000 rpm) at 4 degree for 20 minutes. The supernatants were then collected, aliquoted and stored in -80°C for further analysis. Biopsies were obtained via 18G diagnostic needle and stored in MACS® Tissue Storage Solution (Miltenyi Biotec) at -80°C for further use.

Data source and bioinformatic analysis

Data from TCGA-Liver Hepatocellular Carcinoma (HCC) cohort (<https://portal.gdc.cancer.gov/projects/TCGA-LIHC>) and CNHPP liver data portal (<http://liver.cnhpp.ncpsb.org/>) was retrieved for this study. The gene expression data was collected as raw count values from TCGA data set (135 cases of HBV-positive HCC, 89 cases with no HBV virus infected history). The gene expression, protein and phosphoprotein expression data retrieved from CNHPP liver data portal. R package (edge R) were used for transcriptional profile. The Gene expression data of mRNA, proteome and phosphor-proteome levels were analyzed by t-test. Genes with FDR<0.1 and fold change >1.5 were visualized by TB tool. Pathway enrichment and biological function annotation were performed using Kyoto Encyclopedia of Genes and Genome (KEGG). The differentially expressed gene were analyzed through limma and carried out Gene Set Enrichment Analysis (GSEA) with $p < 0.05$ using the cluster file R package. Data for significant metabolism pathway gene sets were acquired from Molecular Signatures Database (MSigDB <https://www.gsea-msigdb.org/>) and KEGG, and the HCC related activity levels were calculated using ssGSEA. To evaluate the differences in prognosis between the high-risk and low-risk groups, a Kaplan–Meier survival curve was constructed based on the log-rank test.

Protein microarray Fabrication and data processing

The HuProt™ array was constructed from a protein library composed of over 20000 unique human open reading frames using the *Saccharomyces cerevisiae* expression system, each recombinant protein had a GST-His6 tag at the N-terminal [14]. The serum after dilution was added to the array surface. Subsequently, washed with Tris-buffered saline containing 0.1% Tween 20 detergent (TBS-T) for 3 times after incubation for 1 hour. Proceed to the secondary antibodies (anti-human IgG, IgM), after incubation the array was washed and dried in a slide washer (Capital Bio, Inc., Beijing, China), the array was washed and detected with microarray scanner (Capital Bio, Inc., Beijing, China). The raw data, defined as the median foreground and background intensities of every spot in the microarray were download from Scanner. The ratio of foreground to background was regarded as the raw intensity of the spot. The mean signal-to-noise ratio (SNR) was used to represent the signal of the protein. The normalization was carried out by normalize between arrays package in limma [15]. Differentially expressed markers with fold change ≥ 1.2 , and $p < 0.05$, were identified as protein candidates and recruited for further investigation.

Metabolomic analysis

Plasma metabolites separation

Plasma samples (200 μ L) were extracted with 800 μ L of 1:1 (v/v) acetonitrile/methanol. The samples were vortexed and, sonicated for 5 min in ice-water bath. After protein precipitation and centrifuge, the supernatant was collected and dried by vacuum freeze drier (FD-1A-50, Bilon, Shanghai). Reconstituted with 200 μ L acetonitrile/water (1:1, v/v), then processed to LC-MS analysis. Detection of metabolite profile was performed by an AB SCIEX Q-TOF mass spectrometer (X500B) coupled to an ExionLCTM-Ultra High Performance Liquid Chromatography (UHPLC) system (AB SCIEX, Concord, ON, Canada) in both positive and negative ionization. To evaluate the repeatability and robustness of the instrumental system, the quality control process identical to that of the metabolomic analysis was also applied, which was injected every 6 samples. All samples above were maintained at 4°C during preparation and analysis.

Tissue metabolomics and lipidomics sample separation

Tissue metabolites extraction was followed the previous publication [16]. 1.5 mL prechilled methanol/water was added to ~10 mg biopsy for homogenization for 5 min. Centrifuged the mixture at 10000 g for 10 min at 4°C, then collected the supernatant as aqueous extracts. The left pellet was used for organic extracts. Briefly, 1.6 mL prechilled dichloromethane/methanol (3:1) was added to the pellet. After homogenization, the mixture was centrifuged at 10000 g for 10 min, the supernatant was collected as organic extracts. As for the lipidomic sample preparation, biopsy were extracted by liquid-liquid extraction with the MTBE/MeOH/H₂O [17]. 300 μ L cold MeOH was added to each sample followed by homogenization for 2 min, and then 1 mL MTBE was added. The mixture was vibrated then proceed to equilibration at 4°C for 10 mins. Supernatant after centrifuge was collected for further analysis.

UPLC-Q-TOF-MS instrumentation and measurement conditions

Both the hydrophilic interaction liquid chromatography (HILIC) and reverse-phase liquid chromatography were adopted. For reversed-phase separation, a Waters XSelect HSS T3 C18 column (2.1*150 mm, 2.5 μ m) was adopted, while the autosampler and column oven temperature were maintained at 4°C and 40°C, respectively. Mobile phases for C18 consisted of 2 mM ammonium acetate in 0.05/99.95 formic acid/water (A) and 1/1 acetonitrile/isopropanol (B). The column was eluted at 0.3ml/min using a 5-100% phase B, and the injection volume is 1 μ L. Additionally, an ACQUITY UPLC BEH hilic column (2.1*100 mm, 1.7 μ m, Waters, Milford, MA, USA) was adopted. The column elution process was run at 0.3 mL/min with 10 mM ammonium acetate in 0.1/99.9 formic acid/water as phase A, and 95/5 acetonitrile/water containing 10 mM ammonium acetate and 0.1% formic acid as phase B. The autosampler and column oven temperature were maintained at 4°C and 45°C, the injection volume for all samples is 2 μ L.

A reversed-phase C18 column (2.1 mm*100 mm, 2.6 μ m, Kinetex) was used for the chromatographic separation of lipids. The column temperature was kept at 55°C. A gradient of (A) water/acetonitrile (40:60, v/v) and (B) acetonitrile/2-propanol (10:90, v/v), both containing 10 mM ammonium formate at a flow rate of 0.3 mL/min was used. The elution gradient started with 32% B then linearly increased to 85%

B at 15.5 min, and then 97% B at 15.6 min held for 2.4 min. The gradient was back to 32% B at 18.1 min. The temperature of the sample manager was kept at 10°C. The mass spectrometer was operated with a capillary voltage of 3.5 kV in positive mode and 3.0 kV in negative mode. The capillary temperature was set as 300°C. Aux gas heater temperature was 350°C. IDA auto-MS mode was used for MS/MS spectra collection. Combined using strategies, such as comparing the separated peaks from previous MS runs, mass match from information provided by public available databases, as well as molecular structure prediction based on relationships between metabolites and their corresponding M2 features, the metabolites identification was accomplished. The lipids were identified by automated comparison to chemical reference library standard via software MS-DIAL 4.38.

Antibody array

Protein was extracted from the tissue by using RIPA buffer containing proteinase inhibitor. After homogenize, the mixture was centrifuged at 13000 g for 20 min at 4°C. The supernatant containing the soluble protein was collected. The protein expression of PI3K/AKT/mTOR signaling pathway was detected by Human AKT pathway phosphorylation array C1(RayBio/AAH-AKT-1-8). The raw data, defined as the median foreground and background intensities of every spot in the microarray were download from Scanner. The ratio of foreground to background was regarded as the raw intensity of the spot.

Results

1. Multi-omics validation of the HBV induced lipid metabolism alterations in HCC

Although 738 genes from the retrieved TCGA transcriptional matrix showed statistical differences among the two groups (135 HBV-positive HCC patients vs. 89 HBV-negative patients), the PCA analysis showed no obvious separation correlated with HBV infection (data not shown) consistent with the widely accepted cognition that hepatocarcinoma is genetically and biologically heterogeneous (Fig. S1a, Table. S1). As Table. 2 showed, among the most differentially expressed gene sets, the top 20 of 738 differentially expressed genes including LGALS14, BPIFA1, MDBT1, HHATL, PCSK1, SST, CLCA1, ZNF385D-AS2, SOX1 have been reported the correlation with overall survival. The gene annotation analysis for these differentially expressed genes indicated that steroid metabolic process ($-\log_{10}(P)=8.4$), cholesterol metabolic process ($-\log_{10}(P)=8.3$), sterol metabolic process ($-\log_{10}(P)=7.9$), lipid biosynthetic process ($-\log_{10}(P)=7$) were mainly involved pathways (Fig. 1a), as well as glycerolipid metabolism ($\log_{10}P=-15.4$) observed by protein-protein interaction (Fig. S1b). It is widely accepted that proteomics is superior to transcriptomic data in reflecting gene function. Therefore the proteomic characteristics of HBV-associated HCC were analyzed by data matrix from CNHPP (<http://liver.cnhpp.ncpsb.org/>) hepatocellular carcinoma data portal [18] (Fig. 1b). By comparing 1380 human genes from 55 metabolic pathway of the KEGG database (Table. S2), 104 metabolic genes showed significant difference (Table. S3). Combined the transcriptomic and proteomic level of those metabolic genes, the fatty acid metabolism was significantly influenced (as illustrated in Fig. 1c). The dysregulated metabolism of glucose, glycerolipid and amino acids metabolism was also disturbed by

HBV infection (Fig. S1c-e, Table. S4). The metabolic enzymes involved in fatty acid metabolism, including fatty acid desaturase 1 (FADS1), fatty acid desaturase 2 (FADS2), acyl-CoA thioesterase 1 (ACOT1), acetyl-CoA acyltransferase 1 (ACAA1), also enzymes involved in glycolysis, such as phosphofructokinase (PFKM), fructose-bisphosphate aldolase C (ALDOC), enolase 3 (ENO3), showed a different metabolic phenotype of HBV-positive patients compared with HBV-negative patients.

The serum protein profile of HBV-positive/negative HCC patients were next determined. 165 protein candidates related to gastrointestinal cancers designed to the array were listed (Table. S5), which mainly involved in cell membrane formation, cell adhesion, cellular apparatus membrane of Golgi, endoplasmic reticulum membrane, and exosomes (Fig. S2a). 76 of 165 protein candidates showed a statistical difference among HBV-positive HCC with the HBV-negative patients (Fig. 2a, Table. S6). The protein abundance mainly enriched in membrane signaling transduction (JAK-STAT, tyrosine kinase), also metabolism related pathways such as sphingolipid signaling pathway, phospholipase D signaling, regulation of hormone levels (shown in Fig. 2b). Protein such as HRAS, GSK3, DHCR7, which involved in the primary metabolism pathways ranked as the top candidate proteins when compared HBV-positive patients with HBV-negative counterparts. Gene ontology analysis (Fig. S2b) showed that except for membrane signalling and transduction, the metabolic process and immune response also influenced by HBV infection. Metabolomics profile of 36 HCC patients was sequentially determined, 399 of 802 metabolites showed statistically significant compared the HBV-positive patients with HBV-negative counterparts ($p < 0.05$, as shown in Fig. 2c). The metabolite features of 4 modes were illustrated in Fig. S3. Fig. 2d showed that the differentially expressed metabolites involved in pathways, such as primary bile acid biosynthesis ($p = 3.41 \times 10^{-22}$), steroid hormone biosynthesis ($p = 1.03 \times 10^{-21}$), sphingolipid metabolism ($p = 0.000167$). Consistent with the results from bioinformatic analysis based on transcriptomic and proteomic databases that HBV-associated HCC presented lipids metabolic perturbation, majority of lipid species were altered (as Fig. 2c illustrated).

2. HBV influenced sphingolipid metabolism predicted a poor prognosis of HCC

As the lipids perturbation in HBV-positive HCC (shown in Fig. 2c), we next compared the metabolites changes of each pathways from enrichment analysis separately. Fig. 3a depicted the metabolites changes involved in the primary bile acid biosynthesis, particularly the metabolic process of 7-hydroxycholesterol convert to cholic acid or chenodeoxycholic acid were significantly changed. Most metabolites in the HBV-positive group were upregulated compared with the HBV-negative counterparts. In Fig. 3b metabolites involved in sphingolipids biosynthesis was significantly changed between HBV-positive HCC and HBV-negative HCC. Sphingosine-1-phosphate was elevated significantly in HBV-positive HCC patients, indicated its potential as a biomarker for HBV-positive HCC (Fig. 3c). During the steroid hormone biosynthesis, the producing of aldosterone, cortisol, estradiol, and androsterone were enhanced obviously. Next, the correlation of pathway activity and overall survival were evaluated by ssGSEA. With the median value of risk score as cutoff, HCC patients retrieved from TCGA were divided into a high-risk group ($n = 211$) and a low-risk group ($n = 156$). The activity of primary bile acid biosynthesis pathway and steroid hormone biosynthesis pathway showed a better overall survival ($P < 0.001$, Fig. 3d, Fig. S4).

The sphingolipid metabolism pathway, on the other hand, was correlated with a poor prognosis of HCC (Fig. 3e).

3. Primary bile acid and linoleic acid metabolism were influenced by HBV replication.

By comparing serum protein expression profile among HBV-positive patients who possessing higher virus load (shorted as h-HBV-positive) with patients with lower virus titer (l-HBV-positive patients), 8 proteins with the statistical difference were identified (Fig. 4a). Most of the candidate proteins were involved in the membrane, metabolism, particularly the HRAS in choline metabolism, MUC15 in O-glycan processing, GCK in central carbon metabolism. Those discrimination further pointed out the HBV replication could aggravate the dysregulated metabolic environment of HCC patients, compared to patients with lower virus load. In general, the metabolites belongs to primary bile acid biosynthesis, and linoleic acid metabolism pathways were significantly changed in a virus load dependent manner (Fig. 4b,c). Metabolites in bile acid such as cholic acid, glycocholic acid, taurocholic acid were higher in patients with higher virus load. The secondary metabolites in linoleic acid metabolism, particularly metabolites of linoleate, such as 8(R)-HPODE, 9(S)-HPODE, 10(R)-HPODE, 11(S)-HPODE, 13(S)-HPODE were downregulated in the HBV-positive patients possessing higher virus load (as Fig. 4d&e showed), indicated that HBV replication influenced the bile acid and linoleic acid metabolism.

4. Unsaturated fatty acids were significantly accumulated and the hyperactive PI3K/AKT/mTOR was partially responsible for the metabolic rearrangement in HBV-associated HCC.

The metabolic characteristics within the HCC biopsies were determined. The detailed list of metabolites was included in Fig. 5a, Table S7. Table 3 listed the metabolites abundance between tumor and non-tumor adjacent tissue of HBV-positive HCC patients. Metabolites belongs to bile acid (glycohyodeoxycholic acid, glycocholate, glycodeoxycholic acid) and linoleic acid were consistent with previous observation in serum metabolomics (Fig. 3a). Unsaturated fatty acids, such as linoleic acid, vaccenic acid showed a reducing trend in tumor region of HBV-positive patients compared with nontumor region. Compared the metabolites discrepancies in HBV-positive tumor and HBV-negative tumor, long chain unsaturated fatty acids (trans-vaccenic acid, 9-trans-palmitelaidic acid) were higher compared with HBV-negative tumor tissue. Additionally, palmitoylcarnitine were lower in HBV-positive tumor, indicating that HBV downregulated palmitoylcarnitine abundance (Table. 4). As Table 5 listed, hepatic unsaturated fatty acids content was also higher in HBV-positive HCC non-cancerous tissue compared with HBV-negative NT. Namely, 9Z,12Z-linoleic acid, α -linolenic acid, trans-vaccenic acid, oleic acid, 9-Trans-Palmitelaidic acid, nervonic acid were increased in HBV-positive hepatic compared with that of HBV-negative. Together, by tissue metabolomics the perturbation in bile acid metabolism and linoleic acid caused by HBV was confirmed. Metabolites belong to unsaturated fatty acids were higher in HBV-positive group both tumor (T) and nontumor (NT) -adjacent tissue compared with patients do not have HBV infection history (Fig. 5b).

As the lipids perturbation observed from serum metabolites, lipidomic approach was adopted to observe the metabolic changes within tumor. In detail, lipids belongs to glycerophospholipids showed a distinct

profile in HBV-positive tumor compared with HBV-negative counterpart (Fig. 5c). Other than glycerolipids, the abundance of fatty acids, sphingolipids also showed different changes between the two groups. Briefly, metabolites from phosphatidyl-choline was severely decreased in HBV-positive tumor as well as the paratumor tissue which consistent with the trends in serum, indicating the highly consuming of glycerophospholipids during virus replication (Fig. 5d). Metabolites belongs to phosphoethanolamine were increased in HBV-positive tumor. Ceramides were higher in HBV-positive tumor compared to the adjacent tissue. Previous research reported fatty acids exacerbation HCC tumorigenesis mainly via phosphatidylinositol 3-kinase pathway. Therefore we determined the PI3K/Akt/mTOR in metabolic regulation by the antibody array approach. As illustrated in Fig. 6a, the expression of proteins in PI3K/Akt/mTOR signaling of tumor biopsies was probed by antibody array. The fold change of 4E-BP1, GSK3a, GSK3b, PDK1 in HBV-positive tumor was higher compared with the HBV-negative counterparts. We proposed that HBV could probably influenced the signaling cascades, such Raf-1, PDK1, 4E-BP1, eventually activate the transcription of lipogenic genes, therefore enhance the lipogenesis and lipid metabolism (Fig. 6b).

Discussion

In the current study, combined the transcriptomics and proteomics data from two databases, namely TCGA and CNHPP, with the protein abundance and metabolomic signature from our own datasets of HBV-associated HCC cohort, we determined the metabolic reprogramming taking place in HBV-associated HCC, such as activated sphingolipid metabolism which correlated with poor prognosis of HCC, and the perturbation in linoleic acid metabolism and primary bile acid metabolism signaling pathway in a virus load dependent manner. The tissue metabolome partially verified the results from serum metabolome, and identified the significant elevated unsaturated fatty acids. Additionally, the protein array probed the major energy metabolism signaling pathway showed a significant changes along the PI3/AKT/mTOR signaling cascade (as shown in Fig. 7).

Firstly, HBV resulted in a distinct metabolic signature of HCC to gain survival advantage. And HBV positive and negative HCC should be treated with different interventions. With the development in sequencing-based studies, the genetic landscape of HBV-related HCC has been uncovered comprehensively [19]. However, the effect of HBV on the cancer progression remains unclear. By using the transcriptomic data from TCGA, we obtained 738 DEGs compared HBV-positive HCC patients with HBV-negative HCC patients. Upregulated genes such as LGALS14, BPIFA1, MDBT1, HHATL have been reported for their correlation with the overall survival of HCC patients [20–23], while the decreased PCSK1, SST, CLCA1, ZNF385D-AS2, SOX1 predicted a poor overall survival as previous publications reported [24–26]. Previous publication claimed that the metabolic alterations in all primary liver cancer might be metabolically similar, involved metabolic pathways such as glucose metabolism, nucleotide metabolism, amino acid metabolism and lipid metabolism [27]. Our pathway annotation based on metabolic profile (HBV-positive HCC vs HBV-negative HCC) showed that the metabolic alterations mainly attributed to cholesterol metabolic process ($-\log_{10}(P)=8.3$), lipid biosynthetic process ($-\log_{10}(P)=7$). By observing the *in vitro* metabolites profile, the influence of HBV on tumorigenesis was attributed to induction of central

carbon metabolism, including the hexosamine biosynthesis pathway, phosphatidylcholine synthesis, activation of de novo lipogenesis [28].

Primary bile acid metabolism, steroid hormone and the sphingolipid metabolism were influenced by HBV.

Previous studies have reported not only the elevation of bile acid in HCC patients compared with cirrhosis counterparts, also the induction in HBV-infected mice, showed positive correlation of bile acid with HCC, and the HBV-infected animal model [29, 30]. Pathway annotation of metabolites profile in our studies also suggested that the primary bile acid biosynthesis was of higher activity compared to the HBV-negative group. Moreover, in HBV-positive subgroup possessing higher virus load, the metabolites in the primary bile acid biosynthesis pathway were increased compared to HBV-positive patients who possess lower virus load. Metabolic profile from HBV-positive HCC biopsies also showed the abundance of metabolites in bile acid biosynthesis process, including cholic acid, chenodeoxycholic acid, and related intermittent metabolites (T vs. NT). Moreover, the transportation of bile acids within hepatocyte is highly dependent on Na⁺-taurocholate Co-transporting Polypeptide (NTCP), which was initially known as HBV entry receptor (Molecular regulation of the hepatic bile acid uptake transporter and HBV entry receptor NTCP). All together, these results pointed that bile acid metabolism could be a promising target for HBV-positive HCC patients.

Steroid hormones such as androgens, estrogens has been reported play a pivotal role in the progression of HBV infection and the development of HBV-related HCC, mainly via inducing epigenetic regulation such as the regulation of mRNA levels by microRNAs, DNA methylation [31]. Moreover, liver could regulated the synthesis and bioactivity of steroid hormones, which reversely contribute to the proper liver function [32]. For example, testosterone can influence the hepatic lipid deposition, via regulating lipogenic enzyme [33].

Sphingolipids have been reported could help form cellular membrane, contribute to its flexibility and membrane trafficking [34]. The changes of serum sphingolipids in HBV infection and cancer progression was controversial. Some claimed that the increase of sphingolipid in HBV infection and cancer progression, however others reported that most of the sphingolipids were decreased in HBV-positive HCC patients compared with HBV-associated cirrhosis [35, 36]. In our study, not only the upregulation of sphingolipids was observed in HBV-positive HCC compared with HBV-negative HCC, also the activation of sphingolipid metabolism pathway was correlated with a poor prognosis shown by ssGSEA analysis. Moreover, tissue metabolomics showed that the sphingomyelin was higher in biopsies from HBV infected patients (Fig. 6a), so as the lipid profile showed the perturbation of ceramides (Fig. 6d). The upregulation of sphingolipids might contribute to the HBV-related HCC progression. Elevated PDK1 which has been reported correlated with radiation-resistant of HCC [37], thus the activation of PDK1 in HBV-positive HCC patients probably indicated a poor prognosis.

HBV-positive HCC patients showed an altered lipid composition, and accumulation of unsaturated fatty acids. The up-regulated hexosamine and phosphatidylcholine from a cell model (hepG2.2.15) was observed as the HBV stimulated metabolic changes. However, how liver injury and HCC may be

accelerated in patients with HBV infection, and which metabolic pathways are being identified as potential targets to counteract or treat cancer remains unclear. Our results indicated that HBV-positive HCC patients bearing a unique characteristic particularly in lipids categories, such as fatty acyl, glycerophospholipids, sterol lipids, sphingolipids. The abundance of lipid metabolites belongs to primary bile acid, sphingolipids has been discussed above. Aside from the bile acids and sphingolipids, the glycerophospholipids, such as lysophosphocholine 18:2 was higher in HBV-positive HCC biopsy compared with either the non-cancerous tissue from same patients, or the HBV-negative HCC biopsy. Additionally, phosphorylethanolamine 18 showed decrease in HBV-positive NT compared with HBV-negative NT. The accumulation of unsaturated fatty acids has been reported correlated with tumorigenesis, also could interact with immune regulators, thus predicted a poor prognosis in HCC [38, 39]. As our data showed that unsaturated fatty acids, such as linoleic acid, vaccenic acid showed a reducing trend in biopsy of HBV-positive patients compared with the adjacent noncancerous tissue. Additionally, 9Z,12Z-linoleic acid, α -linolenic acid, trans-vaccenic acid, oleic acid, 9-trans-palmitelaidic acid, nervonic acid were increased in HBV-positive hepatic compared with that of HBV-negative, indicated that the unsaturated fatty acid in tumor microenvironment is essential for cancer progression [40].

PI3K/Akt/mTOR resulted lipogenesis partially responsible for metabolic alteration in HCC. As one of the most frequently dysregulated signalling pathway, PI3K/AKT signaling, could stimulate growth, proliferation and survival. The intricate regulation of lipid homeostasis of PI3K/AKT has been reported, not only limited to intracellular process, such as membrane synthesis, but also remodeling the tumor microenvironment by paracrine mechanism [41]. Upon PI3K α recruited to plasma membrane, sequentially phosphorylated phosphatidylinositol (4,5)-bisphosphate and phosphatidylinositol (3, 4,5)-bisphosphate, and subsequent activation of AKT, which ultimately promote the stability of SREBP1c and lipogenesis. As the antibody array showed a hyperactivated PI3K/AKT signaling, particularly the upregulation of 4E-BP1 which involved in the mTORC1 dependent glycolytic pathway, consistent with previous publication about Hepatitis B virus (HBV) pre-S2 mutant transgenic HCC mice model (4E-BP1, a multifactor regulated multifunctional protein). Additionally, the alternative regulation of mTOR, was the GSK3 β promoted 4E-BP1 phosphorylation [42]. The induction of Raf1 by HBV has been reported previously, the activation of Raf/MEK/ERK signaling play a key role in HCC tumorigenesis and development [43, 44]. Our results showed upregulation of Raf1 in biopsy of HBV-positive HCC further indicated Raf1 as a promising target for HBV-HCC patients.

By multi-omics platforms, the metabolic phenotype of HCC, has been extensively investigated [45, 46]. Recently, by linking the metabolites and protein interaction two HCC subtypes were identified, the poor prognostic subtype showed more fatty acid accumulation, however there is no significant difference of HBV infection history among the two groups [39]. These development on omics based approaches have improved our recognition in cancer metabolism, but existing technique can not quantitatively and qualitatively reveal the whole landscape of the metabolism until now. Further experiments should be carried out in the future to validate the detailed metabolic reprogramming and the regulation mechanism.

Conclusion

We investigated the role of HBV replication for promoting HCC progression. The transcriptomic and proteomic results from database combined with our comprehensively protein and metabolome observation, determined the effect of HBV on HCC except for the conventional notion of DNA insertion but also epigenetic regulation, as well as metabolic reprogramming. Taken together, our work pointed out that the role of HBV on hepatocarcinogenesis and tumor progression, particularly related to lipid metabolism (steroid hormone biosynthesis, primary bile acid metabolism, linoleic acid metabolism). The replication of HBV probably influenced metabolites belongs to bile acid metabolism and linoleic acid. Aside from the consistent trend in metabolic profile of biopsy from HBV-positive HCC patients, the lipid composition showed a dramatic changes in biopsies, particularly changes in saturated fatty acid. Ultimately, the changes of lipid metabolism could partially attributed to the hyperactivated PI3K/AKT/mTOR signaling cascade.

Abbreviations

HBV: Hepatitis B Virus; HCC: hepatocellular carcinoma; TCGA: The Cancer Genome Atlas program; CNHPP: Chinese Human Proteome Project; UPLC-ESI-Q-TOF-MS: ultra-performance liquid chromatography coupled with electrospray ionization/quadrupole time-of-flight mass spectrometry; KEGG: Kyoto Encyclopedia of Genes and Genome; GSEA: Gene Set Enrichment Analysis; SNR: signal-to-noise ratio; HILIC: hydrophilic interaction liquid chromatography; FADS1: fatty acid desaturase 1; FADS2: fatty acid desaturase 2; ACOT1: acyl-CoA thioesterase 1; ACAA1: acetyl-CoA acyltransferase 1; PFKM: phosphofructokinase; ALDOC: fructose-bisphosphate aldolase C; ENO3: enolase 3.

Declarations

Ethics approval and consent to participate

This study protocol was reviewed and approved by the Ethics Committee of the Fudan University Shanghai Cancer Center, Shanghai, China (approval no. 1904200-13). Written informed consent was obtained from each participant in accordance with institutional guidelines.

Consent for publication

not applicable

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Competing interests

None declared.

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Not applicable.

Author Contributions

YS and ZM contributed to the conception and design; YZ and ZN contributed to provision of study material or patients; YZ, YD and BL collected and assembled data. YS, ZM and YZ contributed to the data analysis and interpretation. YS, YZ and ZN wrote the manuscript. All authors finally approved the manuscript.

References

1. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021 Dec;7(1):6.
2. Chagas AL, Felga GEG, Diniz MA, Silva RF, Mattos AA, Silva RCMA, et al. Hepatocellular carcinoma recurrence after liver transplantation in a Brazilian multicenter study: clinical profile and prognostic factors of survival. *Eur J Gastroenterol Hepatol*. 2019 Sep;31(9):1148-56.
3. Vogel A, Rimassa L, Sun H-C, Abou-Alfa GK, El-Khoueiry A, Pinato DJ, et al. Comparative Efficacy of Atezolizumab plus Bevacizumab and Other Treatment Options for Patients with Unresectable Hepatocellular Carcinoma: A Network Meta-Analysis. *Liver Cancer*. 2021;10(3):240-8.
4. Magon KL, Parish JL. From infection to cancer: how DNA tumour viruses alter host cell central carbon and lipid metabolism. *Open Biol*. 2021 Mar;11(3):210004.
5. Sangineto M, Villani R, Cavallone F, Romano A, Loizzi D, Serviddio G. Lipid Metabolism in Development and Progression of Hepatocellular Carcinoma. *Cancers (Basel)*. 2020 May 31;12(6):e1419.
6. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009 May 22;324(5930):1029-33.
7. Joo E-J, Chang Y, Yeom J-S, Ryu S. Hepatitis B virus infection and decreased risk of nonalcoholic fatty liver disease: A cohort study. *Hepatology*. 2017 Mar; 65(3):828-835.
8. Ho DW-H, Lo RC-L, Chan L-K, Ng IO-L. Molecular Pathogenesis of Hepatocellular Carcinoma. *Liver Cancer*. 2016 Oct;5(4):290-302.

9. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. *Trends in Biochemical Sciences*. 2010 Aug;35(8):427-33.
10. Currie E, Schulze A, Zechner R, Walther TC, Farese Jr R. Cellular Fatty Acid Metabolism and Cancer: Cell Metabolism. 2013 Aug 6;18(2):153-61.
11. Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N, Koike K. Lipid Metabolic Reprogramming in Hepatocellular Carcinoma. *Cancers (Basel)*. 2018 Nov 15;10(11):447.
12. Liu B, Fang M, He Z, Cui D, Jia S, Lin X, et al. Hepatitis B virus stimulates G6PD expression through HBx-mediated Nrf2 activation. *Cell Death Disease*. 2015 Nov 19;6(11):e1980.
13. Yue D, Zhang Y, Cheng L, Ma J, Xi Y, Yang L, et al. Hepatitis B virus X protein (HBx)-induced abnormalities of nucleic acid metabolism revealed by (1)H-NMR-based metabolomics. *Sci Rep*. 2016 Apr 14;6:24430.
14. Jeong JS, Jiang L, Albino E, Marrero J, Rho HS, Hu J, et al. Rapid identification of monospecific monoclonal antibodies using a human proteome microarray. *Mol Cell Proteomics*. 2012 Jun;11(6):O111.016253.
15. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*. 2003 Jan 22;19(2):185-93.
16. Want EJ, Masson P, Michopoulos F, Wilson ID, Theodoridis G, Plumb RS, et al. Global metabolic profiling of animal and human tissues via UPLC-MS. *Nature Protocols*. 2013 Jan;8(1):17-32.
17. Xuan Q, Hu C, Yu D, Wang L, Zhou Y, Zhao X. Development of a High Coverage Pseudotargeted Lipidomics Method Based on Ultra-High Performance Liquid Chromatography–Mass Spectrometry. *Analytical Chemistry*. 2018 Jun 19;90(12):7608-7616.
18. Jiang Y, Sun A, Zhao Y, Ying W, Sun H, Yang X, et al. Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. *Nature*. 2019 Mar;567(7747):257-261.
19. Gao Q, Zhu H, Dong L, Shi W, Chen R, Song Z, et al. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. *Cell*. 2019 Oct 3;179(2):561-577.e22.
20. Cho Y, Lee YB, Lee J-H, Lee DH, Cho EJ, Yu SJ, et al. Modified AS1411 Aptamer Suppresses Hepatocellular Carcinoma by Up-Regulating Galectin-14. *Plos One*, 2016 Aug 5;11(8):e0160822.
21. Sentani K, Oue N, Sakamoto N, Arihiro K, Aoyagi K, Sasaki H, et al. Gene expression profiling with microarray and SAGE identifies PLUNC as a marker for hepatoid adenocarcinoma of the stomach. *Mod Pathol*. 2008 Apr;21(4):464-75.
22. Frau M, Simile MM, Tomasi ML, Demartis MI, Daino L, Seddaiu MA, et al. An expression signature of phenotypic resistance to hepatocellular carcinoma identified by cross-species gene expression analysis. *Cell Oncol (Dordr)*. 2012 Jun;35(3):163-73.
23. Liu Y, Wang H, Yang W, Qian Y. Prediction of Specific Subtypes and Common Markers of Non-Small Cell Lung Cancer Based on Competing Endogenous RNA Network. *Med Sci Monit*. 2020 Jul 13;26:e922280.

24. Yang B, Cao L, Liu J, Xu Y, Milne G, Chan W, et al. Low expression of chloride channel accessory 1 predicts a poor prognosis in colorectal cancer. *Cancer*. 2015 May 15;121(10):1570-80.
25. Zhang Z, Wang S, Liu Y, Meng Z, Chen F. Low lncRNA ZNF385D-AS2 expression and its prognostic significance in liver cancer. *Oncol Rep*. 2019 Sep;42(3):1110-24.
26. Tsao C-M, Yan M-D, Shih Y-L, Yu P-N, Kuo C-C, Lin W-C, et al. SOX1 functions as a tumor suppressor by antagonizing the WNT/ β -catenin signaling pathway in hepatocellular carcinoma. *Hepatology*. 2012 Dec;56(6):2277-87.
27. Satriano L, Lewinska M, Rodrigues PM, Banales JM, Andersen JB. Metabolic rearrangements in primary liver cancers: cause and consequences. *Nat Rev Gastroenterol Hepatol*. 2019 Dec;16(12):748-66.
28. Li H, Zhu W, Zhang L, Lei H, Wu X, Guo L, et al. The metabolic responses to hepatitis B virus infection shed new light on pathogenesis and targets for treatment. *Sci Rep*. 2015 Feb 12;5:8421.
29. Oehler N, Volz T, Bhadra OD, Kah J, Allweiss L, Giersch K, et al. Binding of hepatitis B virus to its cellular receptor alters the expression profile of genes of bile acid metabolism. *Hepatology*. 2014 Nov;60(5):1483-93.
30. Chen T, Xie G, Wang X, Fan J, Qiu Y, Zheng X, et al. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteomics*. 2011 Jul;10(7):M110.004945.
31. Montella M, D'Arena G, Crispo A, Capunzo M, Nocerino F, Grimaldi M, et al. Role of Sex Hormones in the Development and Progression of Hepatitis B Virus-Associated Hepatocellular Carcinoma. *Int J Endocrinol*. 2015;2015:854530.
32. Charni-Naten M, Aloni-Grinstein R, Osher E, Rotter V. Liver and Steroid Hormones—Can a Touch of p53 Make a Difference? *Front Endocrinol (Lausanne)*. 2019 Jun 12;10:374.
33. Kelly DM, Nettleship JE, Akhtar S, Muraleedharan V, Sellers DJ, Brooke JC, et al. Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice. *Life Sci*. 2014 Jul 30;109(2):95-103.
34. Merrill AH. Sphingolipid and Glycosphingolipid Metabolic Pathways in the Era of Sphingolipidomics. *Chemical Reviews*. 2011 Oct 12;111(10):6378-422.
35. Miura K, Nagahashi M, Prasoon P, Hirose Y, Kobayashi T, Sakata J, et al. Dysregulation of sphingolipid metabolic enzymes leads to high levels of sphingosine-1-phosphate and ceramide in human hepatocellular carcinoma. *Hepatol Res*. 2021 May;51(5):614-26.
36. Wu T, Zheng X, Yang M, Zhao A, Li M, Chen T, et al. Serum lipid alterations identified in chronic hepatitis B, hepatitis B virus-associated cirrhosis and carcinoma patients. *Sci Rep*. 2017 Feb 15;7:42710.
37. Bamodu OA, Chang H-L, Ong J-R, Lee W-H, Yeh C-T, Tsai J-T. Elevated PDK1 Expression Drives PI3K/AKT/MTOR Signaling Promotes Radiation-Resistant and Dedifferentiated Phenotype of Hepatocellular Carcinoma. *Cells*. 2020 Mar 18;9(3):e746

38. Qin X-Y, Su T, Yu W, Kojima S. Lipid desaturation-associated endoplasmic reticulum stress regulates MYCN gene expression in hepatocellular carcinoma cells. *Cell Death Dis.* 2020 Jan 27;11(1):66.
39. Chen D, Zhang Y, Wang W, Chen H, Ling T, Yang R, et al. Identification and Characterization of Robust Hepatocellular Carcinoma Prognostic Subtypes Based on an Integrative Metabolite-Protein Interaction Network. *Adv Sci (Weinh).* 2021 Jul 11;e2100311.
40. Ackerman D, Simon MC. Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. *Trends Cell Biol.* 2014 Aug;24(8):472-8.
41. Koundouros N, Poulogiannis G. Reprogramming of fatty acid metabolism in cancer. *Br J Cancer.* 2020 Jan;122(1):4-22.
42. Shin S, Wolgamott L, Tcherkezian J, Vallabhapurapu S, Yu Y, Roux PP, et al. Glycogen synthase kinase-3 β positively regulates protein synthesis and cell proliferation through the regulation of translation initiation factor 4E-binding protein 1. *Oncogene.* 2014 Mar 27;33(13):1690-9.
43. Pollicino T, Cacciola I, Saffioti F, Raimondo G. Hepatitis B virus PreS/S gene variants: pathobiology and clinical implications. *J Hepatol.* 2014 Aug;61(2):408-17.
44. Li L, Zhao G-D, Shi Z, Qi L-L, Zhou L-Y, Fu Z-X. The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC. *Oncol Lett.* 2016 Nov;12(5):3045-50.
45. Bidkhori G, Benfeitas R, Klevstig M, Zhang C, Nielsen J, Uhlen M, et al. Metabolic network-based stratification of hepatocellular carcinoma reveals three distinct tumor subtypes. *Proc Natl Acad Sci U S A.* 2018 Dec 11;115(50):e11874-83.
46. Zhang X, Li J, Ghoshal K, Fernandez S, Li L. Identification of a Subtype of Hepatocellular Carcinoma with Poor Prognosis Based on Expression of Genes within the Glucose Metabolic Pathway. *Cancers (Basel).* 2019 Dec 14;11(12):2023.

Tables

Table 1
Clinical characteristics of recruited subjects.

Characteristics	Health Control	HBV-positive HCC	HBV-negative HCC
n	28	49	15
age	45.04 $\pm$ 12.06	56.82 $\pm$ 11.70	53.60 $\pm$ 9.78
Sex (male)	28	49	15
AFP	-	226 $\pm$ 34.46	116.1 $\pm$ 58.36
HBV	-	49	-
AST	-	69.28 $\pm$ 11.03	28.6 $\pm$ 3.704
ALT	-	86.27 $\pm$ 9.627	38.31 $\pm$ 6.110
CA-199	-	66.56 $\pm$ 24.58	132.4 $\pm$ 63.94

Table 2
Top 20 upregulated and downregulated genes.

Upregulated			Downregulated		
Gene names	logFC	p-value	Gene names	logFC	p-value
MT1B	10.2328	2.62E-16	PCSK1	-7.53782	3.68E-62
GPR50	7.780407	1.64E-14	SST	-7.33738	2.78E-14
TNP1	6.892576	1.18E-10	CLCA1	-7.25852	2.92E-18
STRC	6.230527	6.87E-24	AC152010.1	-6.30304	1.67E-17
TRARG1	6.143218	7.01E-15	PHOX2A	-6.24551	1.24E-17
RNU6ATAC	5.890879	8.29E-22	NELL1	-6.00629	2.86E-16
RNU6-554P	5.266137	1.84E-11	ZNF385D-AS2	-5.55186	9.45E-12
AC009312.1	5.045021	7.25E-10	PAK5	-5.43078	9.87E-17
LGALS14	5.022113	3.75E-09	SOX1	-5.41698	2.14E-16
LINC02054	4.988967	1.92E-14	MOS	-5.37088	6.73E-14
BPIFA1	4.969714	4.54E-08	MUC2	-5.22148	9.12E-25
EDDM3B	4.891035	6.70E-09	FADS2B	-5.1755	1.73E-13
PNLIP	4.888525	9.37E-10	FGF4	-5.09179	2.01E-12
AC009646.2	4.738481	1.09E-06	METTL21C	-5.00554	5.92E-13
KC877982.1	4.709765	5.33E-07	DLGAP1-AS5	-5.00535	3.68E-16
MT1CP	4.704529	1.25E-15	AL136140.1	-4.9734	1.28E-11
AC021393.1	4.699334	2.62E-08	AC109780.1	-4.89645	1.30E-17
C16orf89	4.528714	1.70E-21	DMRTB1	-4.88103	8.39E-13
TPRKBP1	4.496049	1.03E-09	AC104793.1	-4.86705	4.86E-14
AC243972.2	4.362346	1.74E-10	LINC02583	-4.80742	4.33E-12

Table 3
Metabolites changes in HBV-positive HCC patients (T vs. NT).

Metabolites	Fold change	
Myristic acid	1.22	1.14
9Z,12Z-Linoleic acid	0.91	0.62
Trans-Vaccenic acid	0.56	0.72
LPC 18:2	1.29	1.75
Palmitoylcarnitine	0.75	0.27
Cholic acid	0.80	0.73
Glycohyodeoxycholic acid	4.08	3.01
Glycocholate	3.51	1.28
Glycodeoxycholic acid	3.77	2.82

Table 4
Metabolites changes between tumor tissue of HBV-positive patients and HBV- negative counterparts.

Metabolites	Fold change	
Trans-Vaccenic acid	1.78	1.15
LPC 18:2	1.93	1.13
Phosphatidylethanolamine 16	0.51	0.93
Cholesterol	5.18	2.67
Palmitoylcarnitine	0.45	0.15
9-Trans-Palmitelaidic acid	3.28	1.19

Table 5
Metabolites changes between paratumor tissue of
HBV-positive patients and HBV-negative
counterparts.

Metabolites	Fold change	
Palmitic acid	2.82	1.23
9Z,12Z-Linoleic acid	1.56	1.48
Trans-Vaccenic acid	4.05	1.67
Lignoceric acid	0.51	0.50
Phosphatidylethanolamine 18	0.11	0.59
α -Linolenic acid	0.75	0.89
Cholesterol	11.22	1.42
Deoxycholic acid	1.16	1.21
Palmitoylcarnitine	0.64	0.66
Oleic acid	3.61	1.46
9-Trans-Palmitelaidic acid	2.28	1.39
Nervonic acid	6.87	1.91
Glycohyodeoxycholic acid	0.20	0.08
Glycocholate	0.23	0.16
Glycodeoxycholic acid	0.18	0.11
Inosine	0.43	0.46

Figures

Figure 1

Transcriptional differences between HBV-positive HCC and HBV-negative HCC patients. a. Gene annotation analysis for thes differentially expressed genes (DEGs) between HBV-positive and HBV-negative HCC patients that retrieved from TCGA database (HBV-positive HCC=135, HBV-negative HCC=89). b. The chord diagram indicated the top differentially expressed mRNAs (HBV-positive vs. HBV-negative) from CNHPP and the accordingly biological functions involved. c. Changes of fatty acid metabolism based on the expression of mRNAs, protein and phosphopeptide data of CNHPP matrix.

Figure 2

Protein profile of HCC patients with different HBV infection status. a. The volcano plot illustrated the differentially expressed serum protein candidates between HBV-positive HCC and HBV-negative counterparts. Green dots indicated the downregulated proteins, red dots stand for the upregulated. b. Heatmap showed the function annotation of the 74 significantly different protein candidates among HBV infected HCC and HCC without HBV by metascape, as indicated that most proteins participated in signaling transduction, such as JAK-STAT, Tyrosine kinase signaling pathway. c. Metabolites heatmap showed the metabolites profile discrimination mainly attributed to lipid species, particularly fatty acyl, glycerolipids. d. Metabolomic pathway analysis via MetaboAnalyst. Circle stands for the enriched pathway. The radius of the circle represented the impact of the pathway.

Figure 3

The discrepancy of metabolic processes and its influence on overall survival. a. Discremination of metabolites involved in primary bile acid biosynthesis (HBV-positive vs. HBV-negative). b. The metabolites discrepancies took place in sphingolipid biosynthesis pathways (HBV-positive vs. HBV-negative). c. The different metabolites belongs to steroid hormone biosynthesis pathway (HBV-positive

vs. HBV-negative). d&e. Kaplan–Meier survival analyses of high-risk and low-risk groups in the TCGA cohort associated with fatty acid metabolism (d), sphingolipid metabolism (e).

Figure 4

Influence of lipid metabolism between HBV-negative and HBV-positive patients. a.Heatmap showed the differential of protein abundance between h-HBV-positive patients and l-HBV-positive patients. b&c. The illustration showed the changes of metabolites that belongs to priamry bile acid biosynthesis process (b) and linoleic acid metabolic process (c). d&e. Statistical analysis of the metabolites discrepancies in the priamry bile acid biosynthesis process (d) and linoleic acid metabolism (e).

Figure 5

The metabolites change of HCC patients. a. The metabolic characteristics of tumor biopsies of HBV-positive HCC patients compared with adjacent tissue (NT), and the HBV-negative HCC patients. b. Paired changes of metabolites (relative expression T vs. NT) between HBV-positive patients and HBV-negative. c. Lipids changes in HBV-positive HCC patients compared with their counterparts, both the abundance in tumor biopsies (T), as well as the adjacent region (NT). d. Paired lipid changes (relative expression T vs. NT) in HBV-positive patients and their counterparts.

Figure 6

The PI3/Akt/mTOR pathway changes in HBV-related HCC probed by antibody array. a. Image and statistical analysis of antibody array to show the changes of PI3/Akt/mTOR signaling cascade of biopsies obtained from HBV-related HCC patients. b. Illustration the major changes probed of PI3/Akt/mTOR.

Figure 7

Schematic diagram of the study. a. The design of the study, firstly the transcriptomics and proteomics data from public database were used to observed the metabolic discrimination between HBV-positive and HBV-negative HCC patients, then by proteome and metabolome screening of our own cohort, the dysregulated metabolic pathways was confirmed. Next, the biopsies was probed using antibody array, which furtherly verified the underlying mechanism. b. The HBV replication induced metabolism changes

within HCC patients, including the upregulation in fatty acid biosynthesis, increased bile acid, enhanced lipogenesis and the down regulation of linoleic acid, which probably regulated by the activated PI3K/AKT signaling cascades. The upregulated fatty acid biosynthesis, resulted in the elevated glycerophospholipids and sphingolipids which could promote the membrane formation sequentially HBV replication, and signaling transduction.

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