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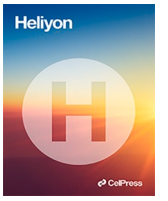

Research article

Apolipoprotein H-based prognostic risk correlates with liver lipid metabolism disorder in patients with HBV-related hepatocellular carcinoma

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ABSTRACT

Background: /Aim: Chronic hepatitis B patients often develop concomitant fatty liver disease, which is associated with increased risk of liver cirrhosis and hepatocarcinoma. Our previous studies have shown that apolipoprotein H (APOH) levels are gradually decreased in patients with chronic HBV infection at different stages of disease progression, and APOH deficiency disrupted hepatic lipid metabolism and caused fatty liver. We focus on the relationship between APOH and hepatocellular carcinoma (HCC) in the context of chronic HBV infection.

Methods and results: *APOH* was downregulated at the transcriptional level in HBV-related HCC patients from open-source human liver transcriptome databases, and relatively high expression of *APOH* might be a favourable prognostic marker in HCC. *APOH* downregulation was positively associated with tumour grade and HCC subtypes. The analysis result of CHCC-HBV database showed that *APOH*-associated differential expression genes (DEGs) enriched in lipid metabolic pathways and downregulated APOH correlated with macrophage, neutrophil and CD8 T cell infiltration levels. Next, *in vitro* experiments were performed and *APOH* gene was silenced in HepG2.2.15 cells, an HBV producing human HCC cells. Further transcriptomic assay and analysis revealed the DEGs were enriched in cholesterol metabolism. The subsequent RT-qPCR experiments identified that *CYP7A1* expression was higher upregulated in APOH silencing HepG2.2.15 cells than vehicle control cells ($p < 0.05$). Finally, demographic data of patients with HBV-related HCC were enrolled, and serum APOH levels were analysed using ELISA. Serum APOH levels were significantly lower in patients with HCC than in healthy controls ($p < 0.05$), and positively correlated with triglyceride level in healthy controls ($p < 0.05$). In HBV-HCC patients, serum APOH levels were positively correlated with albumin levels and negatively correlated with alkaline phosphatase (ALP), total bilirubin, and INR levels ($p < 0.05$).

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Conclusion: APOH downregulation disrupted liver lipid metabolism to potentially affect the overall survival in patients with HBV-related HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer worldwide. In China, HCC is the most frequently caused by chronic HBV infection [1]. However, the increasing prevalence of metabolic disorders for HCC has been the predominant proportion in the number of cases of HCC in the future. Recent studies report that nearly one quarter of patients with chronic hepatitis B (CHB) also have concurrent hepatocyte steatosis, which leads to the development of active hepatitis, liver cirrhosis, and HCC in these patients [2–5]. However, the mechanisms underlying the associations between hepatocyte steatosis and HCC derived chronic HBV infection are still unclear.

Apolipoproteins are responsible for lipid transport and metabolism in liver. Recently, multiple studies demonstrate the interaction of apolipoproteins with classical pathways of tumorigenesis and might serve as potential biomarkers for cancer patients [6]. The previous study primarily focused on the correlations between APOH and HBV infection and elucidated that HBV and its large surface antigen directly upregulates APOH expression, which inhibits HBsAg secretion and induces hepatocyte ER stress [7,8]. Further studies clarified that *ApoH* gene knockout mouse appears spontaneous steatohepatitis and APOH deficiency disrupted hepatic lipid metabolism and gut microbiota homeostasis [9,10]. Based on these complied results, APOH might be a hinge point in fatty liver disease incidence and progression during chronic HBV infection and we focus on this direction in the present study.

This study explored the correlation of APOH and HCC in the context of chronic HBV infection. Firstly, the expression of APOH in liver tumour tissues was analysed and the prognostic value of APOH in HBV-related HCC patients was calculated. To further investigate the *APOH* gene-associated liver transcriptome, liver metabolic and immune microenvironment in the CHCC-HBV database. Next, serum APOH levels were measured to understand their potential correlations in patients with HBV-related HCC. This study shed light on the possible role of APOH in HBV-related HCC and highlights that downregulation of APOH-induced abnormal hepatic lipid metabolism may be associated with poor patient prognosis.

2. Materials and methods

2.1. Expression of APOH and survival analyses from online liver tumour databases

The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), International Cancer Genome Consortium (ICGC), and Chinese HCC patients with HBV infection (CHCC-HBV) databases were used to analyze the transcriptional expression levels of *APOH* in paired tumour and non-tumour liver tissues. Patients were further divided into two subgroups based on median value of *APOH* expression (high vs. low expression), and Kaplan–Meier survival plots, hazard ratios, and log-rank *P*-values were calculated to compare the two subgroups. Additionally, the expression of *APOH* in HCC was further analysed for correlations with sex, age, tumour grades, or body mass index (BMI). All analysis was performed using R software (version 4.2.2).

2.2. In silico analysis of APOH-associated liver microenvironment in the CHCC-HBV database

Next, APOH-associated consensus subtypes of HCC and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using CHCC-HBV database. The Spearman coefficients of the APOH were calculated, whilst correlation >0.3 and $p < 0.05$ were defined as *APOH*-related genes in the present study. Gene set enrichment analysis (GSEA) was performed in the *APOH*-related differential expression genes (DEGs). The fold change of gene expression between the tumour group and non-tumour group was calculated, and the gene list (*APOH*-related DEGs) was generated according to the change of $\log_2$ FC. The results were generated and visualized by using the R package cluster Profiler, ggplot2, and Enrich plot. Additionally, the immune estimation analysis was performed using the estimation tools on CIBERSORT [11], microenvironment cell populations-counter (MCP-counter) [12], and xCell [13]. The ESTIMATE algorithm was used to calculate the fractions of immune cell types in R software (version 4.2.2). Macrophage estimation was further analysed by investigating the difference in infiltration based on *APOH* expression.

2.3. Cell culture and transfection

HepG2.2.15 cell is a stable HBV-producing hepatoma cell line, which was gifted by Academy of Military Medical Sciences (Beijing, China). The cells were cultured in completed DMEM (Hyclone, Waltham, MA, USA) with 10 % Fetal Bovine Serum (FBS), 1 % penicillin/streptomycin (Gibco, Grand Island, NY, USA), and 380 μg/ml Geneticin® G418 sulfate (Gibco).

HepG2.2.15 cells were seeded in 6-well plates and cultured overnight to reach 50 %–60 % confluence for further transfection. Lipofectamine™ 3000 transfection reagent (Invitrogen, Waltham, MA, USA) was used to perform the transient transfections. Next, cells were seeded in 6-well plates and transfected with 30 pM small interfering RNA (siRNA), which were mixed with Lipofectamine RNAiMAX (Thermo Fisher Scientific, Waltham, MA). After incubation for 24–36 h, the transfected cells were washed and used for the following experiments. The human *APOH* siRNAs (SMART pool) and non-targeting siRNAs were purchased from Dharmacon (GE

Table 1
Primers used in RT-qPCR analysis.

Gene		Primers (5′- 3′)
GAPDH	Forward	AATCCCATCACCATCTTCCA
	Reverse	TGGACTCCACGACGTACTCA
APOH	Forward	GCCCATCAACACTCTGAAATG
	Reverse	CGCCATTCAGATAAAACCCAG
CYP7A1	Forward	AATTTCACTTTGCTACTTCTGCG
	Reverse	TGAGGGAATTCAAGGCATGG

Table 2
Demographic data and serum markers of patients in this study.

Variable	Healthy Control (N = 12)	Hepatocarcinoma (N = 12)	*P*-Value
Gender (F/M)	3/9	1/11	0.284
Age in yrs	55.17 ± 11.32	55.25 ± 12.70	0.977
BMI (kg/m2)	22.96 ± 2.79	23.35 ± 2.46	0.908
HBV DNA (IU/ml)	–	200.00(200.00,8874.00)	–
HBsAg (IU/ml)	0.10(0.10,0.10)	189.91(100.00,356.97)	–
ALT (U/L)	20.50(16.35,24.20)	45.35(28.65,54.85)	0.003
AST (U/L)	22.50(19.15,27.00)	40.25(37.60,59.20)	<0.001
GGT (U/L)	26.75(21.30,39.90)	74.00(46.80,101.85)	<0.001
ALP (U/L)	79.20(68.05,87.20)	113.45(94.35,156.35)	<0.001
pre-ALB (g/L)	–	83.64(75.44,91.83)	–
ALB (g/L)	41.97 ± 1.49	38.39 ± 6.02	0.083
TBiL (μmol/L)	14.35(13.20,16.80)	17.70(14.35,40.45)	0.094
DBiL (μmol/L)	2.60(2.05,3.05)	3.85(3.15,10.20)	0.004
IBiL (μmol/L)	12.00(10.35,13.75)	14.10(11.15,31.00)	0.204
TBA (μmol/L)	–	63.73(27.19,100.27)	–
CHE (U/L)	–	3319.50(2288.00,4351.00)	–
TG (mmol/L)	1.22(1.03,2.00)	0.92(0.62,1.36)	0.078
TC (mmol/L)	5.66 ± 0.86	4.36 ± 1.04	0.007
HDL (mmol/L)	1.30 ± 0.32	1.06 ± 0.27	0.112
LDL (mmol/L)	3.75 ± 0.62	2.92 ± 0.82	0.018
LP (a) (mg/L)	–	33.15(17.90,48.40)	–
Apo-A1 (g/L)	1.37 ± 0.26	1.02 ± 0.30	0.005
Apo-B (g/L)	1.07 ± 0.17	0.82 ± 0.21	0.005
BUN (mmol/L)	5.10(4.60,5.55)	5.30(4.35,6.35)	0.773
CRE (μmol/L)	70.08 ± 13.48	65.60 ± 14.40	0.488
GLU (mmol/L)	5.63 ± 1.05	6.53 ± 2.46	0.391
Plt (X10^9/L)	209.00(181.00,261.50)	182.50(86.00,240.50)	0.141
INR	0.99 ± 0.06	1.16 ± 0.18	0.004
AFP (ng/ml)	2.74(2.12,5.17)	112.50(4.85,903.60)	0.005

Healthcare).

2.4. Real-time quantitative PCR and RNA-sequencing analysis

HepG2.2.15 cells were silenced with APOH expression and then extracted total RNA using the commercial RNeasy kit (QIAGEN, Germantown, MD) in accordance with the manufacturer's instructions. The detailed real-time quantitative PCR (RT-qPCR) procedures have been described in previous studies [8,10]. The primers used are listed in Table 1. Simultaneously, samples were selected to run transcriptome assay by Beijing Genomics Institute (BGI) (N = 2) and then analysed using BGI automatic analysis software. The KEGG enrichment pathways were identified, which was calculated using the Term Candidate Gene Num/Term Gene Num. A Q-value of $\leq$0.05 showed significant enrichment.

3. Clinical sample acquisition

3.1. Plasma specimens

The current cross-sectional study was performed to investigate the potential functions of serum APOH in patients with HBV-related HCC, who received therapy at Xiamen University Zhongshan Hospital in southeast China. The enrolled patients were diagnosed and treated according to clinical practice guidelines [14–17]. Patients were excluded with other types of chronic liver diseases such as hepatitis C or autoimmune liver disease. Clinical information was obtained from medical records. Demographics (age, sex, body mass index [BMI]), serum biochemical indicators (serum lipid levels, liver function, renal function, coagulation function), clinical diagnoses, imaging, antiviral therapeutic strategies, and concomitant diseases were compiled to display cohort features. In total, 12

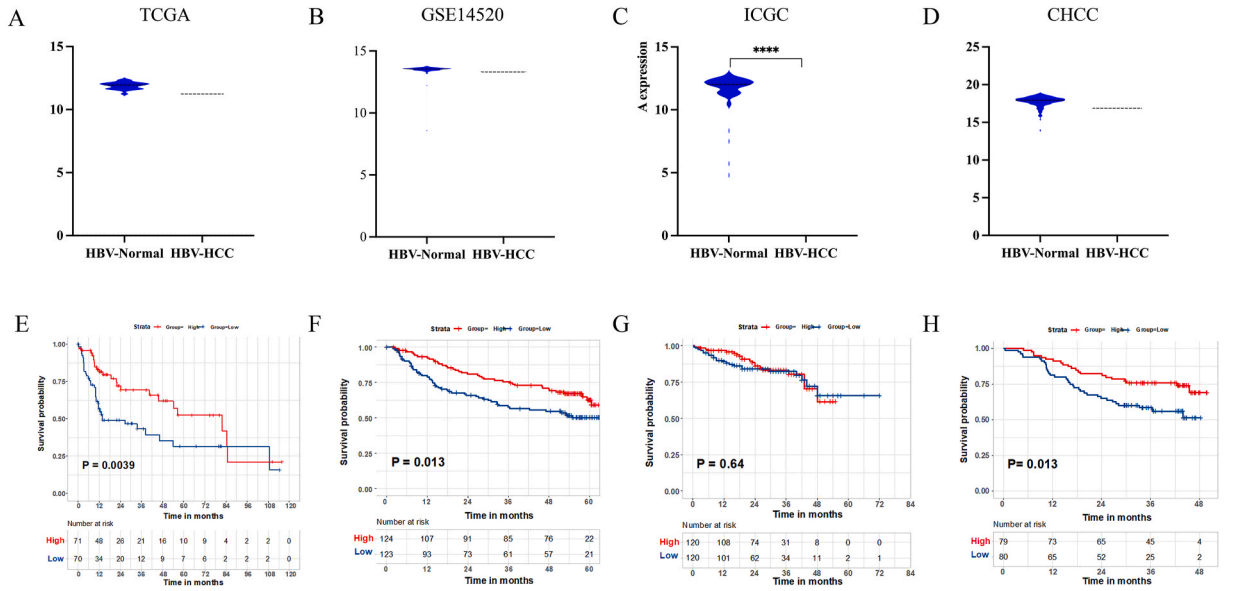

Fig. 1. *APOH* expression in LIHC and its effect on patient survival **(A–D)** *APOH* expression was analysed at the transcriptional level in patients with HBV-related HCC and paired non-tumour liver tissues in the TCGA, GEO (GSE14520), ICGC, and CHCC-HBV databases, respectively. *APOH* expression was significantly downregulated in the liver tumor of HBV-HCC patients compared with nontumor controls ($p < 0.0001$).
(E–H) Corresponding overall survival curves based on *APOH* expression in HBV-HCC patients from the TCGA, GEO (GSE14520), ICGC, and CHCC-HBV databases, respectively. The survival time was longer in HBV-HCC patients with relatively high APOH expression than low APOH expression group ($p < 0.05$).

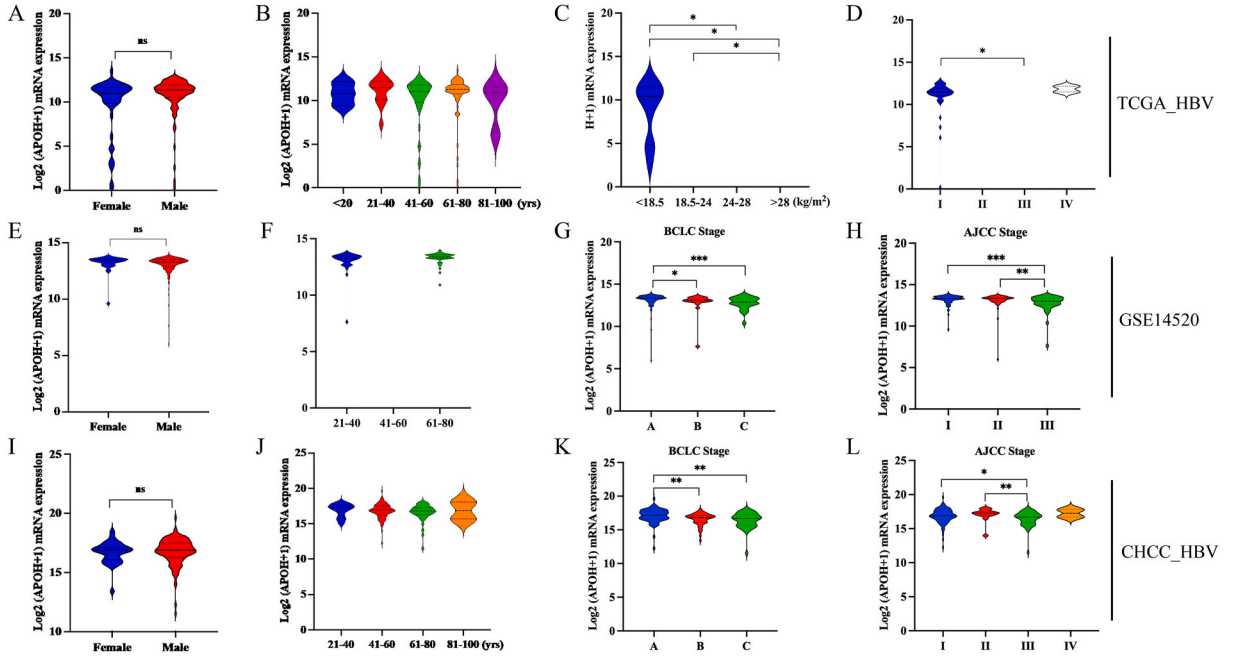

Fig. 2. Association of APOH expression with tumour grade and HCC subtypes **(A, E and I)** Sex differential *APOH* expression in HBV-HCC patients from TCGA, GEO (GSE14520) and CHCC-HBV databases. **(B, F and G)** Age differential *APOH* expression in HBV-HCC patients from TCGA, GEO (GSE14520) and CHCC-HBV databases. **(C)** *APOH* expression at different BMI index of HBV-HCC patients from TCGA database. Normal weight: 18.5 ≤ BMI <25; extreme weight: 25 ≤ BMI <30; obese: 30 ≤ BMI <40; extremely obese: BMI >40. **(G and K)** *APOH* expression in HBV-HCC patients based on BCLC tumour stages. **(D, H and L)** *APOH* expression in HBV-HCC patients based on AJCC tumour stages. Tumours are graded as 1, 2, 3, or 4, depending on severity. In Grade 1 tumours, tumour cell morphology, and organization of the tumour tissues appear close to normal. These tumours tend to grow and spread slowly. In contrast, Grade 3 and Grade 4 tumours tend to grow rapidly and spread faster than tumours with a lower grade. *$p < 0.05$; **$p < 0.01$.

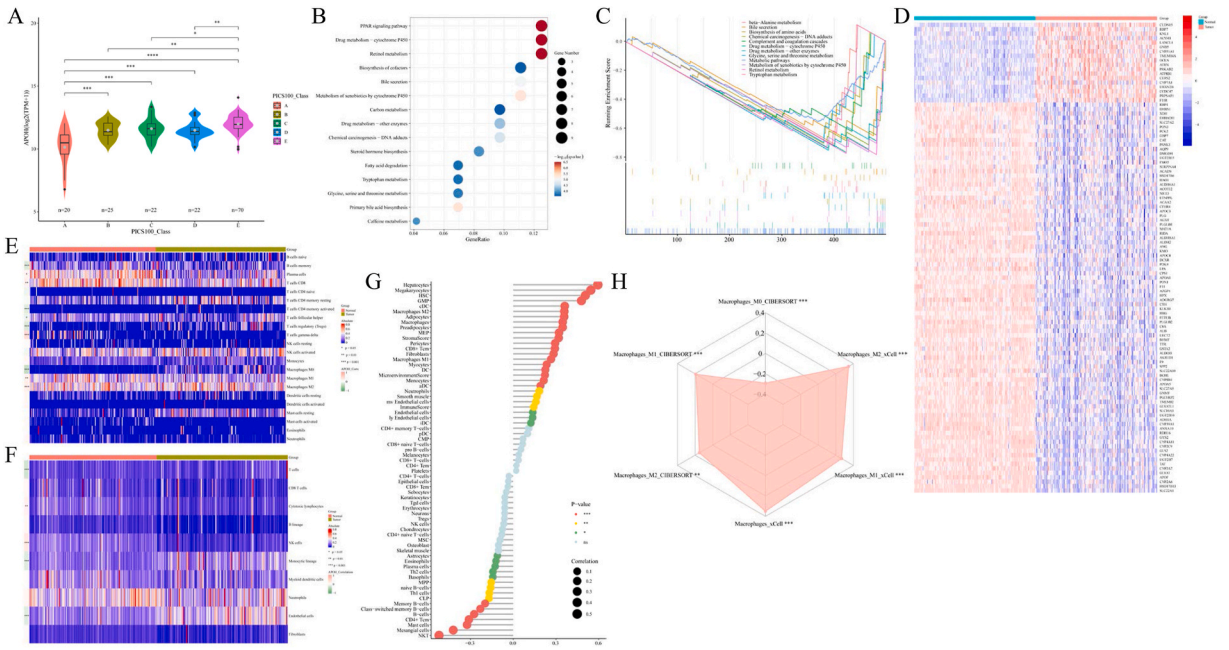

Fig. 3. APOH expression and associated transcriptomic analysis in the CHCC-HBV database **(A)** *APOH* expression at the transcriptional level was significantly lower in Subtype A tumours than other subtypes and was the highest in Subtype E tumours. HBV-HCC is divided into five distinct subtypes according to clinical and biological homogenous. **(B)** *APOH*-associated genes were identified and analysed the enriched signalling pathways in the KEGG databases. **(C)** *APOH*-associated downregulated genes were identified and analysed the enriched signalling pathways by GESA database. **(D)** The heatmap showed the *APOH*-associated DEGs (correlation >0.3, $p < 0.05$, $\log_2$ FC = 1). **(E–G)** The abundance analysis of *APOH*-associated immune cells infiltration using CIBERSORT (E), MCP-count (F), and xCell (G) algorithms. **(H)** *APOH*-associated M0, M1 and M2 macrophage infiltration level in liver tumour microenvironment.

patients with HBV-associated HCC and 12 healthy persons were included in this study (Table 2).

3.2. Human serum APOH ELISA assay

Serum samples were collected and stored at −80 °C freezers until test and analysis. Serum APOH levels (80,000-fold dilution) were quantified using an Abcam ELISA kit (Cat#274669, Abcam, Cambridge, MA, USA).

3.3. Statistical analysis

A non-parametric test was used to display the skewed distribution of sample data. Kruskal–Wallis tests showed the differences between two groups, followed by Mann–Whitney *U*-tests (two-tailed). Correlations were analysed using Spearman rank correlation. Results were described as the mean ± standard error of mean (SEM). The results of two-tailed Student's *t*–test or analysis of variance (ANOVA) indicated the statistical significance between different group. *P* value is less than 0.05 to be considered statistically significant.

4. Results

4.1. APOH expression in HBV-related HCC and its effect on patient survival

First, the expression of *APOH* was compared in paired tumour and non-tumour liver tissues in the TCGA, GEO, ICGC, and CHCC-HBV databases. *APOH* expression at the transcriptional level was decreased in patients with HBV-related HCC (Fig. 1A–D). The corresponding survival analysis indicated that overall survival (OS) was significantly longer in patients with higher *APOH* expression ($p < 0.05$, Fig. 1E, F, and 1H; $p > 0.05$, Fig. 1G). Further analysis showed that *APOH* expression in patients with HBV-HCC was related to tumour grade, patient weight, and other factors. And *APOH* expression gradually decreased with the progress of tumor grading and staging in the TCGA (Fig. 2D), GEO (Dataset: GSE 14520; Fig. 2G and H), and CHCC (Fig. 2K and L) databases ($p < 0.05$). Furthermore, *APOH* expression obviously enhanced with the increasing of BMI index in the TCGA-HBV database ($p < 0.05$; Fig. 2C). Significant differences in *APOH* expression were observed between 41 and 60 years of age and 61 years or older in the GEO database ($p < 0.05$; Fig. 2F). However, age had no significant effect on *APOH* expression in the TCGA-HBV (Fig. 2B) and CHCC-HBV (Fig. 2J) databases. And sex had no significant in all the three databases (Fig. 2A, E and 2I). Above all, the relatively high expression of *APOH* may be a

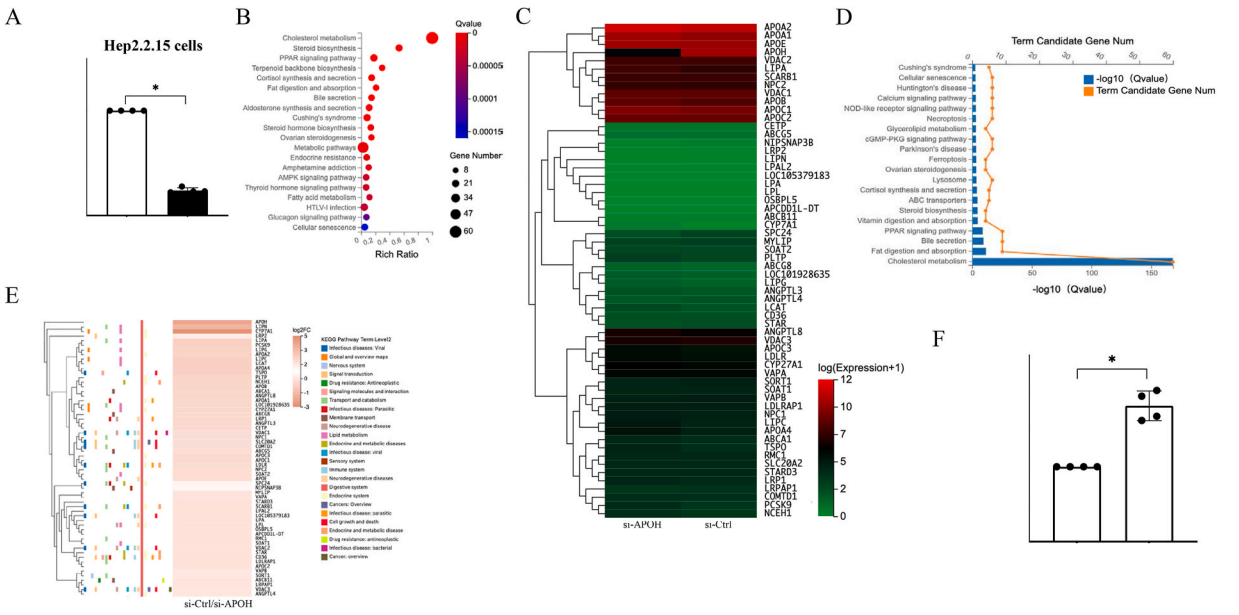

Fig. 4. RNA-seq analysis from APOH-silencing HepG2.2.15 cells **(A)** *APOH* mRNA expression in HepG2.2.15 cells were detected using RT-qPCR. **(B)** The bubble chart showed the cholesterol metabolism related pathways by KEGG pathway analysis. **(C)** Cholesterol metabolism related DEGs (52 genes) was illustrated by a heatmap. **(D)** The enrichment analysis of 52 DEGs was performed by KEGG database analysis. **(E)** Relative expression of genes involved in regulating process in the *APOH* silencing *versus* control cells. **(F)** *CYP7A1* mRNA expression was detected by RT-qPCR. Results are presented as the mean ± SEM. $*p < 0.05$.

favourable prognostic marker in liver cancer.

4.2. *APOH expression and associated transcriptomic analysis in the CHCC-HBV database*

Further analysis clarified the correlations between *APOH* expression and consensus HCC subtypes in the CHCC-HBV database [18]. *APOH* expression was significantly lower in Subtype A tumours than other subtypes, while it was highest in Subtype E tumours (Fig. 3A). To test a possible involvement of APOH gene in the liver microenvironment, the relative genes expression and the corresponding pathways and as well as immune infiltration in silico were analysed.

First, the *APOH*-associated gene enrichment pathways by KEGG database analysis were illustrated in Fig. 3B and mainly included "PPAR signalling pathway", "Bile secretion", "Fatty acid degradation", "Primary bile acid biosynthesis", and Cytochrome P450-related metabolism pathways. The heatmap indicated the *APOH*-related DEGs (Fig. 3D). The *APOH*-associated downregulated genes were predominantly enriched in "Bile secretion" and some metabolic pathways using GSEA analysis (Fig. 3C).

Next, the absolute abundance and proportion of *APOH*-associated immune cells were calculated to describe the liver immune microenvironment. Among the three algorithmic approaches, the CIBERSORT algorithm was used to analyze the proportions of 22 types of immune cells (Fig. 3E). The infiltration levels of macrophage and T cells were closely correlated with *APOH* expression. MCP-counter method quantified the absolute abundance of 8 immune and 2 stromal cell population using liver transcriptomic data (Fig. 3F). The infiltration levels of monocyte and neutrophil cells were closely correlated with *APOH* expression. xCell is a new gene signature-based method and was calculated to infer 64 immune and stromal cell types (Fig. 3G). A negative association was observed between *APOH* expression and macrophages, monocytes, CD8 T, DC, adipocytes, and fibroblast cells infiltration levels, whereas *APOH* expression was positively associated with CD4 T cells, mast cells, B cells and NKT cells infiltration levels. *APOH*-associated macrophages infiltration level was calculated, which was notably and positively related to total macrophage levels in liver microenvironment. No significant difference was observed between M1 and M2 macrophage infiltration levels (Fig. 3H). Taken together, *APOH* expression may be essential for metabolic and subsequent immune regulation in the abovementioned liver microenvironment.

4.3. *RNA-seq data analysis from APOH-silencing HepG2.2.15 cells*

To explore the transcriptomic regulatory of silencing *APOH* expression on HepG2.2.15 cells, RNA-seq analysis was performed in this study. *APOH* mRNA expression was showed in Fig. 4A. Our data revealed that *APOH* gene was mainly associated with lipid metabolism as illustrated by KEGG pathway analysis in Fig. 4B and *APOH*-related DEGs (52 genes), including *CYP7A1*, *CYP27A1*, *CD36*, apolipoproteins and many other genes, was indicated by heatmap (Fig. 4C). Further analysis indicated 52 DEGs were enriched in cholesterol metabolism (Fig. 4D) and *CYP7A1* was identified the top differential expression gene in *APOH*-silencing HepG2.2.15 cells (Fig. 4E). *CYP7A1* mRNA expression was further verified to be obviously increased in the *APOH* silencing group by RT-qPCR method (*p*

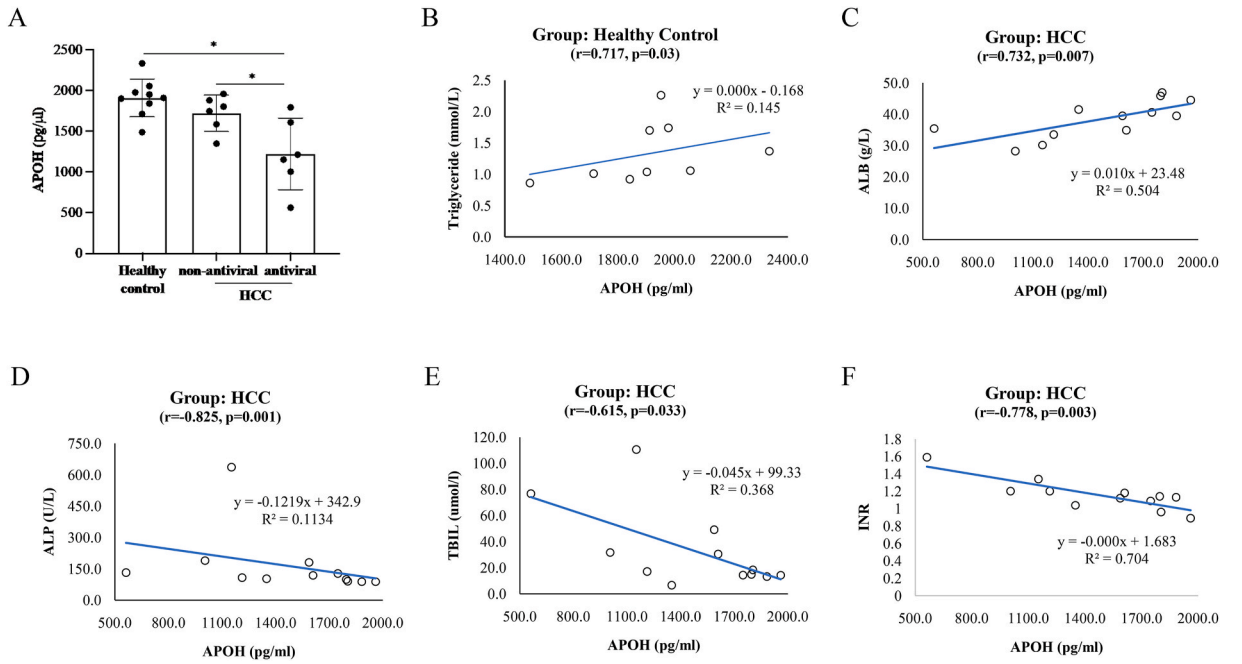

Fig. 5. Analysis of APOH levels in patients with HBV-related HCC **(A)** Serum APOH levels were detected using ELISA in patients with HBV-HCC, who were classified into two subgroups based on antiviral therapy use. **(B)** Correlations between serum APOH and triglyceride levels in the healthy control group ($p < 0.05$). **(C–F)** Correlations between serum APOH levels and ALB, ALP, TBIL and INR levels in HBV-related HCC patients ($p < 0.05$).

< 0.05) (Fig. 4F). Overal, APOH production decreased in the context of HBV related HCC, which caused aberrant lipid metabolism, especially cholesterol metabolism.

4.4. APOH levels and associated clinic analysis in patients with HBV-related HCC

Then, the associations between serum APOH levels and HBV-related HCC were focused on in this study. Clinical characteristics are presented in Table 2. Both groups had a median age of 55 years. Most HCC patients (11; 91.67 %) were male. No significant sex-based differences were observed in patients ($p > 0.05$). No obvious difference in BMI was observed between the groups, and the median BMI in each group was within the normal range. Serum APOH levels were significantly lower in patients with HCC, especially in patients treated with antiviral drugs, than in healthy controls ($p < 0.05$; Fig. 5A). Serum APOH levels were positively associated with triglycerides level in healthy controls ($p < 0.05$; Fig. 5B). Moreover, serum APOH levels were positively correlated with ALB levels and negatively correlated with ALP, TBIL, and INR levels in HCC patients (Fig. 5C–F; $p < 0.05$). Therefore, APOH was mainly associated with liver reserve function, bilirubin metabolism, and coagulation function in patients with HBV-related HCC.

5. Discussion

The framework of this study was deciphered by a graphical abstract (Fig. 6). This study firstly analysed the correlations between APOH and HBV-related HCC using open-source databases. The main results were as follows: 1) *APOH* expression was downregulated at the transcriptional level in patients with HBV-related HCC and associated with tumour grade and HCC subtypes; 2) relatively *APOH* high expression in HCC patients predicted more favourable prognosis; and 3) *APOH*-associated aberrant metabolic activation was the predominantly enriched biological process in liver tumour tissues from patients with HBV-related HCC. Above all, APOH might be a hinge point in fatty liver disease progression during chronic HBV infection and might also be an indicator of favourable prognosis in HBV-related HCC.

Further analysis elucidated the correlations between *APOH* and other encoding genes from liver tumour tissues in the CHCC-HBV database. Lipid metabolism pathways was the predominant enriched biological processes and *CYP7A1* was the preferred related genes which involved in the regulation process. Of note, isoenzymes *CYP7A1* primarily participated in steroid and cholesterol biosynthesis and bile acid metabolism [19]. The above findings have been further validated with the *in vitro* experimental results by APOH-silencing HepG2.2.15 cells. In summary, APOH production downregulated in the context of HBV-related HCC, which activated aberrant lipid metabolism pathways and chiefly affected steroid hormone and bile acid biosynthesis.

Additionally, the liver microenvironment homeostasis was disrupted in patients with chronic liver diseases (CLDs), which affected liver cell function, caused metabolic and immune dysregulation, and eventually facilitated the progress of CLDs [20]. Thus, further

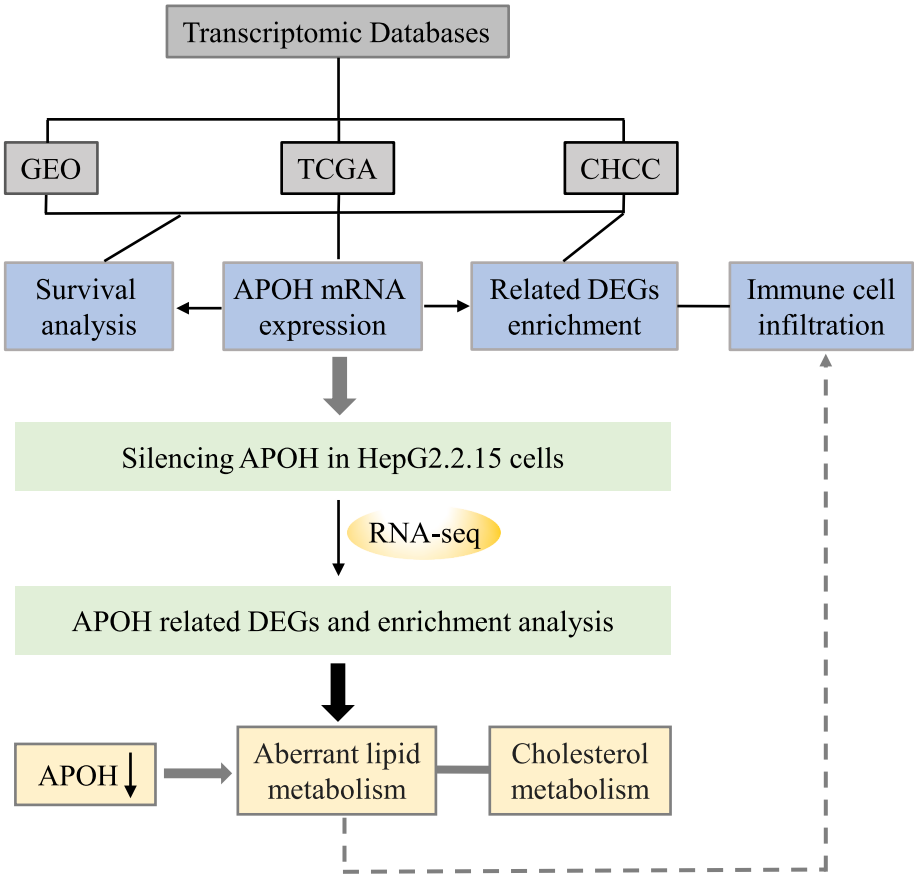

Fig. 6. The research framework of apolipoprotein H-associated aberrant lipid metabolism in patients with HBV-related hepatocellular carcinoma. This study analysed the correlations between APOH and HBV-related HCC using open-source databases and the findings provide the proof-of-concept on HBV-related HCC being associated with APOH-mediated lipid metabolism dysregulation and subsequent immune microenvironment remodelling.

study assessed the association of *APOH* expression with immune cell infiltration using CHCC-HBV database. We found that *APOH* was notably and positively related to total macrophage levels in liver microenvironment. Macrophages were functionally classified into "pro-inflammatory" M1 phenotype and the alternatively activated "immunoregulatory" M2 phenotype [21]. In the CHCC-HBV database, no obvious difference was observed between M1 and M2 macrophage infiltration levels using the ESTIMATE and CIBERSORT algorithms. Overall, it is possible that with the progression of chronic HBV infection, reduced APOH level dysregulated lipid metabolism and subsequent impacted macrophages infiltration levels in the abovementioned liver microenvironment.

Finally, the associations of serum APOH levels with liver function, lipids levels, and coagulation function was summarized and analysed in patients with HBV-related HCC. Serum APOH levels notably decreased in patients with HCC, which was consistent with the database analysis. We also found APOH was closely associated with liver reserve function, bilirubin metabolism, and coagulation function in patients with HBV-related HCC. From STRING website, functional protein association networks showed that APOH co-expressed with apolipoproteins and coagulation genes. Some studies reported that APOH participated in the transportation and metabolism of triglyceride and cholesterol in liver and was a key regulator of antiphospholipid syndrome [22]. Together, the findings were confirmed that APOH was downregulated and disrupted hepatic lipid metabolism in patients with HBV-related HCC.

In summary, CHB and fatty liver were the focus of the present research effort. This study identified the potential functions of APOH during chronic HBV infection and the findings provide the proof-of-concept on HBV-related HCC being associated with APOH-mediated lipid metabolism dysregulation and subsequent immune microenvironment remodelling. All the results also suggest that APOH might be an indicator of favourable prognosis in HCC. Importantly, this study constructs a strong research framework for further studies evaluating the role of APOH in the process of CLDs, which is favourable for transforming to clinic as a valuable diagnosis biomarker and accurate treatment target.

6. Study strengths and limitations

The present study primarily clarified the APOH level and associated analysis at transcriptional level in HBV-related HCC patients using the multiple open-source databases. The results showed that downregulated APOH predominantly impaired the liver lipid

metabolism to cause hepatocyte steatosis during the progress of CLDs, and subsequently might affect liver immune cells infiltration which further facilitated the development of hepatocellular carcinoma. The limit clinical patient samples were used to confirm the conclusions in this study, to further enlarge the sample size and supplement the histopathologic and prognostic data in the future study.

Data availability

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

This study was carried out following the Declaration of Helsinki and approved by the Ethics Committees of Zhongshan Hospital Xiamen University (No. xmzsyyky2020145). Informed consent was obtained from all study participants.

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CRediT authorship contribution statement

Yaming Liu: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zeyi Wu:** Investigation. **Yiqun Zhao:** Investigation. **Maochuan Zhen:** Resources. **Yanhong Wang:** Formal analysis, Data curation. **Qiusong Liu:** Resources.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yaming Liu has patent issued to Yaming Liu. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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