American Journal of Biochemistry and Biotechnology

Research Paper

Dihydroartemisinin Attenuates HBV-Induced Proliferation and Migration in LO2 cells via EMT Pathway Modulation

Yingde Nong[1], Caifang Ma[2], Wei Huang[1], Yichong Ning[3], Shanbin Chen[4], Xueqing Guo[1], Huihua Deng[3], Yongqiang Wei[3], Wenmei Zhao[3]

[1]Department of Clinical Laboratory, The People's Hospital of Chongzuo, Chongzuo, China;
[2]Medical Science Laboratory, Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Nanning, China;
[3]Chongzuo Biomedical Clinical Transformation Key Laboratory, The People's Hospital of Chongzuo, Chongzuo, China;
[4]International Joint Research Center for Liquor Quality and Safety, Beijing, China

Article history
Received: 24 March 2025
Revised: 12 May 2025
Accepted: 17 June 2025

*Corresponding Author:
Wenmei Zhao
Chongzuo Biomedical Clinical Transformation Key Laboratory, ChongZuo, China;
Email: wenmeizhao77@163.com

Abstract: Dihydroartemisinin (DHA), a derivative of artemisinin, possesses well-documented anti-inflammatory properties. This study investigated the inhibitory effects of DHA on hepatitis B virus (HBV)-induced cellular alterations in LO2 cells and elucidated underlying molecular mechanisms. Serum interleukin-6 (IL-6) levels were measured in HBV patients and analyzed for correlations with HBsAg, anti-HBs, and HBeAg. *In vitro*, LO2 cells were infected with clinical HBV serum samples (1 PEIU/ml, 72 hours) and treated with DHA (10 μM). Cell phenotypes were assessed using viability (MTT assay), proliferation (colony formation assay), and migration (scratch wound and Matrigel invasion assays). Protein expression related to proliferation (IL-6, tumor necrosis factor-α [TNF-α], programmed death ligand-1 [PD-L1]), apoptosis (Bcl-2 [B-cell lymphoma-2], Bax [Bcl-2-associated X protein]), cell cycle (p21 [cyclin-dependent kinase inhibitor 1A], cyclin D1), and epithelial-mesenchymal transition (E-cadherin, α-catenin, N-cadherin) were quantified by Western blotting. Serum IL-6 positively correlated with HBsAg/HBeAg and negatively correlated with anti-HBs. HBV infection increased cell viability, proliferation, and migration capacity, and upregulated IL-6, TNF-α, PD-L1, Bcl-2, cyclin D1, and N-cadherin protein expression, while downregulating Bax, p21, E-cadherin, and α-catenin. DHA treatment suppressed HBV-induced malignant phenotypes and reversed these protein expression changes. These findings demonstrate that HBV activates an IL-6/TNF-α/PD-L1 signaling axis to drive malignant transformation, while DHA attenuates this process through multi-target inhibition, supporting its potential therapeutic application in HBV-associated liver disease.

Keywords: Dihydroartemisinin, Hepatitis B Virus, Epithelial-Mesenchymal Transition, Cell Proliferation, Cell Migration, Anti-Inflammatory Agents, IL-6, PD-L1

Introduction

Chronic hepatitis B virus (HBV) infection continues to pose a significant global health challenge, with approximately 5~10% of acute cases progressing to chronic stages. Viral replication triggers inflammation, which, in turn, sustains chronic damage leading to cirrhosis and hepatocellular carcinoma (Yuen *et al.,* 2018). Typically, compromised immune defenses cause an increase increases the expression of tumor necrosis factor α (TNF-α) (Zahara *et al.,* 2024). TNF-α induces the expression of immune checkpoint programmed death ligand-1 (PD-L1) through the nuclear factor κ-B (NF-κB) signaling pathway (Wang *et al.,* 2017). Altered PD-L1 expression not only promotes immune evasion but also influences cellular proliferation, metastasis (Lan *et al.*, 2022; Yang *et al.,* 2021), and drug resistance (Meng *et al.,* 2021). Furthermore, Interleukin-6 (IL-6) induces the expression of PD-L1 in hepatocellular carcinoma via the Janus Kinase 1 pathway (Chan *et al.,*). Nevertheless, the impact of IL-6 and TNF-α on initiating epithelial-mesenchymal transition (EMT), a critical step in cancer metastasis, in non-cancerous liver cells remains largely uncharted territory. IL-6 and TNF-α play pivotal roles across clinical stages of chronic HBV infection and and have been proposed as biomarkers for assessing disease severity (Bekçibaşı *et al.,* 2021). Critically, the mechanistic nexus between HBV-induced inflammation

and hepatocyte malignant transformation remains contentious. Controversy persists regarding whether HBV directly induces EMT. While viral infections typically promote EMT in cancerous cells (Wu *et al.,* 2023), evidence in normal hepatocyte lineages (e.g., LO2 cells) remains limited. Moreover, while PD-L1 facilitates HBV persistence (Huang *et al.,* 2021), its regulation by IL-6/TNF-α and crosstalk with EMT pathways remain unestablished.

Many phytochemical compounds produced by plants are advantageous to health, and many medicines are inspired by the therapeutic effects of plants (Sitio *et al.,* 2024). Compared to synthetically produced drugs, botanical formulations are inherently more natural and safer, with components typically better suited for human absorption. Therefore, explore into novel applications of plant-derived active compounds holds great promise. Dihydroartemisinin (DHA) is a derivative of artemisinin. DHA not only has the characteristics of high efficiency and low toxicity in the field of antimalarial treatment, but also has played multiple important roles in anti-inflammation, anti-tumor and immune regulation in recent years (Dai *et al.*, 2021). DHA can regulate proinflammatory cytokines (e.g., TNF-α and interleukin) and plays an anti-inflammatory role. Chronic inflammation has been implicated in carcinogenesis initiation and progression. DHA inhibits the release of pro-inflammatory factors (such as TNF-α and IL-6) by suppressing the NF-κB and ERK signaling pathways. This mechanism disrupts carcinogenic transformation within inflammatory microenvironments. (Yu *et al.,* 2013; Zhang *et al.,* 2021). The side effects of traditional radiotherapy and chemotherapy (Nobel *et al.,* 2024) stem from their non-selective killing of normal cells. Preclinical studies of DHA have confirmed that its combination with chemotherapy drugs can reduce the dose of the latter by 30 ~ 50%, alleviate side effects such as gastrointestinal reactions and bone marrow suppression (Tao. 2016; Dai *et al.,* 2024). These findings demonstrate DHA's capacity to act as a multi-target drug in blocking inflammation, cancer prevention (inhibiting the transformation from inflammation to cancer), and adjuvant therapy (sensitizing radiotherapy and chemotherapy), demonstrating significant clinical translational value.

LO2 as normal liver cells it usually has no invasiveness. But HBV infection may temporarily alter their phenotype through specific mechanisms such as inflammatory signaling or EMT. We thus hypothesize that HBV exploits certain signaling cascades to drive malignant phenotypes in LO2 hepatocytes, and that DHA can reverse this process. Given DHA's anti-inflammatory properties in combating hepatitis B, this research delved deeper into how DHA and HBV interact in hepatocytes in vitro. This study aimed to uncover the underlying molecular pathways, to explore innovative applications of DHA and to lay a solid scientific foundation for improved prevention and treatment strategies for hepatitis B.

Materials and Methods

Chemicals and reagents

DHA (Aladdin, 81496-82-4), LO2 cells were obtained from the Chinese Academy of Sciences cell bank, Carbon dioxide incubator (Shanghai bosun, bc-j160-s), biosafety cabinet (Biobase, bsc-1500ii b2-x), inverted microscope and imaging system (Olympus, Japan),Flow cytometry (BD FASVia), electrophoresis and membrane transfer tank (Tanon), developer (Tanon, 5200multi), room temperature and low temperature high-speed centrifuge (biobase), metal bath (Thermo, USA), microplate reader (Rayto, rt-6100), scanner (Epson), dihydroartemisinin (Aladdin), FBS, RPMI 1640, penicillin streptomycin double antibody solution, 0.25% trypsin (GIBCO), Transwell chamber (Corning, 3422), antibody: IL-6 (Abclonal, a0286), TNF-α (Abclonal, a11534), PD-L1 (Abclonal, a1645), Bax (CST, 2772), p21 (Abclonal, a1483), bcl-2 (Wanleibio, wl01556), E-cadherin (Abclonal, a3044), N-cadherin (Abclonal, a1483) clonal, a19083), cyclin D1 (Abclonal, a19038), α-catenin (Abclonal clonal, a19004), GAPDH (Abclonal, a19056). BCA, RIPA, MTT, ECL and SDS-PAGE gel preparation kit, etc, were purchased from Shanghai Sangong. PageRuler™ Prestained Protein Ladder, 10 to 180 kDa (Thermo, 26617), etc. Blood samples were tested for IL-6 with the clinical testing instrument cobas e411, and hepatitis B was analyzed with the automatic fluorescence analyzer Easycuta.

Clinical Specimen Collection and Detection

The clinical specimens were collected in People's Hospital of Chongzuo, Chongzuo, Guangxi, China, between January 2021 and December 2021. The level of IL-6 and HBV serological markers (HBsAg, anti-HBs and HBeAg) was detected by Cobas e 411 and by EasyCuta. The Medical Ethics Committee of People's Hospital of Chongzuo approved and reviewed the study. (No. KS202101(01)) according to the principles of Helsinki Declaration.

Cell Culture

LO2 cells were grown in RMPI-1640 medium with 10% FBS and 1% penicillin-streptomycin, then incubated at 37 ℃ with 95% humidity and 5% CO_2. When the cells grew to more than 90%, trypsin was used to digest the cells, the cells were collected into BD tubes, and they

were centrifuged at 1000 revolutions for 3 minutes. Finally, trypsin was removed, fresh complete medium was added, the cells were resuspended, and they were then subculture at 1:3. In vitro, LO2 cells constructed a cellular inflammation model. And investigate the inhibitory effect of DHA on HBV in LO2 cells and its molecular mechanism.

Cell Viability Assay

LO2 cells were grown into 96-well plates at 5000 cells per well and allowed 24 hours to attach. The cells were subsequently assigned to three experimental groups: (i) Mock group (untreated). (ii) HBV-infected group (LO2 cells infected with HBeAg clinical serum medium containing a final concentration of 1 PEIU/ml). (iii) HBV-infected + DHA group (10 μM DHA treatment). At specified time points (Days 1~4), thiazolyl blue tetrazolium bromide (MTT solution, 5 mg/mL in PBS) was added to achieve a final concentration of 10% (v/v), and the plates were incubated for 4 hours at 37 ℃. After incubation, the formed formazan crystals were dissolved by adding 100 μL of DMSO and gently shaking the plate for about 10 minutes. The absorbance was then measured at 490 nm with a microplate reader. Proliferation fold-change was calculated as:

$$Proliferation\ flod-change=\frac{OD_{day\ T}}{OD_{day\ 1}}$$

Three independent experiments were performed in triplicate.

Clone Formation Assay

Cells from each experimental group were plated in 6-well plates at 500 cells per well and maintained in 2 mL of RPMI-1640 medium enriched with 10% FBS. The HBV+DHA group received continuous 10 μM DHA supplemented. Cultivation occurred under standard conditions of 37 ℃, 5% CO_2, and 95% humidity over a period of 15 days. After incubation, the plates were fixed with a 4% paraformaldehyde solution for 15 minutes, then stained with 1% crystal violet for 5 minutes, and the number of colonies was counted.

Cell Cycle Detection Via Flow Cytometry

The distribution of cells cycle was examined using a propidium iodide (PI) staining kit from Multi Sciences (Hangzhou, China), following the instructions provided by the manufacturer. Detection was carried out through Flow cytometry (BD FACSVia).

Scratch Assay

By using a 6 well plate, two parallel lines were drawn with a marker pen in each hole at the bottom, after which cells were counted and then evenly spread in each group in the 6-well plate. There were 1×10^6 cells in each hole, and 9 holes for each type of cell. The cells were grouped according to the abovementioned criteria, mixed evenly by using the cross method, and placed in 37℃ cell incubators. When the cells were confluent, the scar was marked perpendicular to the parallel line with the tip head of 200 μL pipette gun, the desquamated cells were cleaned with PBS, the corresponding medium was added to the groups (without FBS), photos were taken at the intersection of the marking and the scratch, the 24-hour cell migration state was recorded, and the migration ability was analyzed via Adobe Photoshop.

Matrigel Invasion Assay

The matrix containing Transwell chamber was put into the base membrane of the incubator for hydration for 30 minutes. Cells in each group were collected as follows: Lower chamber (500 μL): Add medium containing 10% FBS. Upper chamber (200 μL): a different group of cell suspensions without serum. After 72 hours of culture, the cells were fixed with methanol for 20 minutes and subsequently stained with a 0.1% crystal violet solution for an additional 20 minutes. The excess dye that was non-specifically bound to the upper surface of the cells was carefully removed by wiping the upper side. Following air drying, cell observation and counting were conducted in three fields under a microscope.

Western Blotting

Cells in each group were collected as follows: the supernatant waste liquid was removed, the cells were washed with PBS at 4 ℃ (3 times), the PBS was removed, an appropriate amount of lysate was added into the well, and the protein was collected. After 10 minutes on ice, the samples were centrifuged at 14000 g and 4 ℃ for 10 minutes, and the protein was transferred to a new EP tube. A BCA kit used to measure the protein concentration, and the corresponding steps were performed according to the BCA kit instructions of biotechnology. An appropriate amount of denaturant SDS was added, after which the solution was heated to 105 ℃ with a metal bath for denaturation for 10 minutes. Ten percent of the separation gel was selected, the electrophoresis voltage was set at 100 V, the current was set at 10 mA, and the electrophoresis was conducted for 3 ~ 4 hours. Moreover, 200 mA film rotation was used for 2 hours. Subsequently, 50 mL of 1 × TBS and 5 g skimmed milk powder were used to seal the plate on the shaking table for 1 hour. The primary antibody was applied at 4 ℃ overnight, followed by washing the membrane three times with 50 mL TTBS for 10 minutes each time. Subsequently, the secondary antibody was incubated at room temperature for 1 hour, after which the membrane was washed again three times with 50 mL TTBS for ten minutes per wash. Tianneng

5100 developer was used to develop the films.

Statistical Analysis

All the statistical analyses were performed using Excel 2010 (Microsoft, Seattle, WA, USA). Date Data are presented as the mean ± standard deviation (SD). Intergroup differences were assessed using the Student's t-test. The statistical significance level indicated by * and #. *: HBV group vs. mock group; #: HBV + DNA group vs. HBV group. **/ ## P < 0.01 ; ***/ ### P < 0.001. Sigma plot (SYSTAT software, San Jose, CA) was used to statistically analyze the correlation between IL-6 and HBV.

Results

HBV Upregulates the Expression of IL-6 in Vivo

The detection of IL-6 is recommended as an indicator to evaluate the degree of chronic hepatitis B in generally. Collected 15 clinical HBV positive samples and 22 normal physical examination samples, detected HBV serological markers (HBsAg, HBeAg and anti-HBs) and IL-6, and analyzed the correlation. The results indicated that IL-6 exhibited a positive correlation with HBsAg and HBeAg, and negatively correlated with anti-HBs. The positive correlation between IL-6 and HBeAg (r=0.3484) suggests that viral replication directly fuels inflammation. This result showed that IL-6 can increase after HBV infection, and the expression of IL-6 can decrease when the body produces antibodies (1A-C).

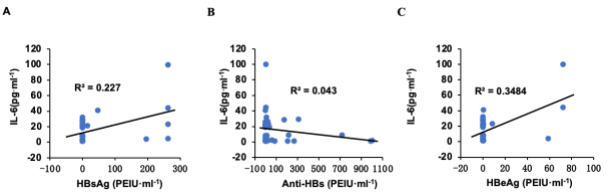

Fig. 1. Collection of clinical specimens. (A) The correlation between IL-6 and HBsAg; (B) The correlation between IL-6 and Anti-HBs; (C) The correlation between IL-6 and HBeAg.

HBV induces dichotomous phenotypes in LO2 cells: proliferation suppression with enhanced migration

In vitro, selected normal human hepatocyte LO2 cells for HBV induction, constructed a cellular inflammation model, and added DHA to the HBV model. Cell viability, colony formation, and cycle analyses were performed in the untreated mock group, HBV group and HBV+DHA group. The results showed that HBV increased viability and clonogenicity (P<0.001) vs. mock group, this paradoxically coincided with G2/M arrest (G1 phase ↓, G2 phase ↑, P<0.01). The G2/M accumulation indicates DNA damage cerheckpoint activation (a hallmark of replication stress induced by HBV X protein), which disrupts cyclin-dependent kinase activity. Compared with the HBV group, the cell viability and clonogenic ability of the HBV+DHA group were significantly decreased (P<0.01). Furthermore, the cell cycle was significantly increased in the G1 phase and was significantly decreased in the G2 phase (P<0.01) (Fig. 2A-C). It showed that HBV induced abnormal proliferation of LO2 cells, whereas DHA can inhibit the proliferation of LO2 cells induced by HBV.

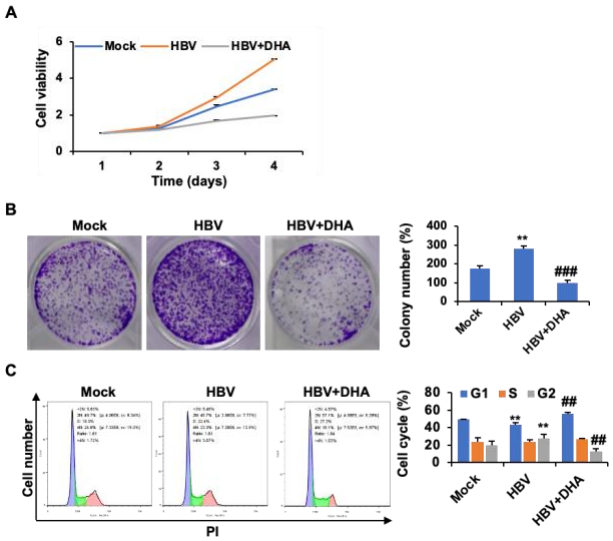

Fig. 2. The effect of DNA treatment on HBV-induced LO2 cells proliferation and cell cycle. (A) Proliferation fold-change. (B) Clonal formation. (C) The cell cycles. *: HBV group vs. mock group; #: HBV + DNA group vs. HBV group. **/## $P < 0.01$; ***/### $P < 0.001$.

DHA Reverses HBV-Driven Oncogenic Phenotypes by Targeting EMT and Apoptosis Regulators

Cell migration and invasion capacities were assessed across three experimental groups using scratch wound healing and matrigel invasion assays. Compared to the Mock group, HBV infection significantly enhanced both migration (P<0.01) and invasion (P<0.01). In contrast, DHA treatment (10 μM) markedly suppressed these HBV-driven phenotypes, reducing migration and invasion versus the HBV group (P<0.001, Fig. 3A-B). These results demonstrate that DHA effectively counteracts HBV-mediated oncogenic progression by regulating EMT.

DHA inhibits HBV-induced proliferation and migration of LO2 cells through IL-6/TNF-α/PD-L1 axis

This study detected three groups of cellular proteins

by using Western Blotting and preliminarily discussed the molecular mechanism by which DHA inhibits HBV-induced LO2 cells proliferation and migration. Our results showed that the content of IL-6//TNF-α/PD-L1 in HBV histones increased significantly; after DHA treatment, the content of IL-6//TNF-α/PD-L1 was significantly decreased. When considering the effects of HBV and DHA on the proliferation and migration of LO2 cells, selected downstream proteins related to proliferation and migration. Our experimental results showed that the expression of bcl-2/cyclin d1/n-cadherin protein was upregulated in the HBV-induced group. Moreover, the expression of bax/p21/e-cadherin/α-catenin protein was downregulated (Fig. 4). This indicated that DHA may pass through IL-6//TNF-α/PD-L1 to inhibit HBV-induced proliferation and migration of LO2 cells. DHA reversal of these changes validates its potential as a multi-pathway inhibitor for HBV-related liver disease.

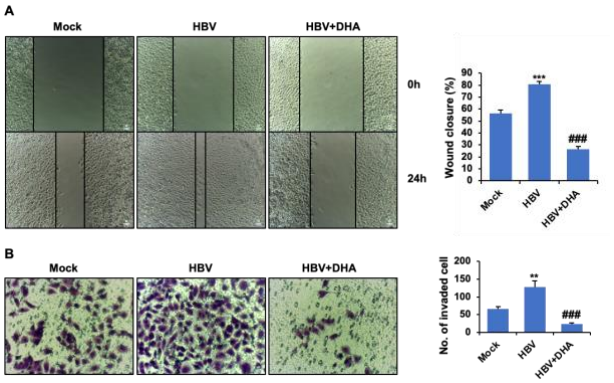

Fig. 3. The effect of DHA treatment on LO2 cells migration. (A) Scratch test. (B) Matrigel invasion tests. *: HBV group vs. mock group; #: HBV + DNA group vs. HBV group. **/## $P<0.01$; ***/### $P<0.001$.

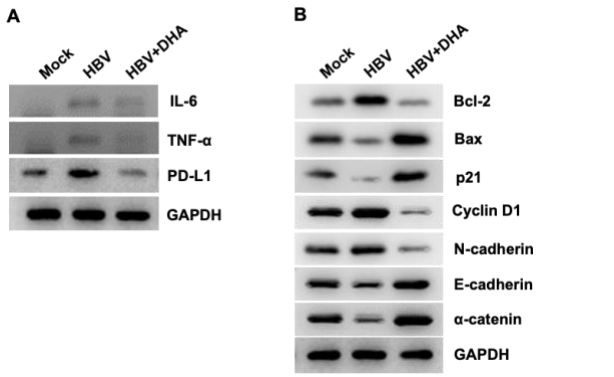

Fig. 4. The effect of DHA treatment on protein expression of LO2 cells.

Discussion

This study reveals a novel IL-6/TNF-α/PD-L1 signaling axis as central to HBV-driven hepatocyte pathogenesis. We demonstrate that HBV-infected LO2 cells significantly up-regulated the protein expression of IL-6, TNF-α, and PD-L1, concomitant with enhanced proliferation and invasion phenotypes. Critically, DHA suppresses these effects and reverses EMT markers (up-regulated E-cadherin/ down-regulated N-cadherin).

Prior studies established viral EMT induction in hepatoma cells ((Ting *et al.,* 2018) showed HBx-mediated Twist1 activation in HepG2), but evidence in normal hepatocytes was lacking. In this study, HBV infected LO2 cells model confirmed HBV-driven cadherin switching (down-regulated E-cadherin/up-regulated N-cadherin) and enhanced matrix invasion phenotypes. This finding extends prior work in HBV-associated hepatocarcinoma models suggested that HBV can initiate malignant pro-transformation in normal liver cells through PD-L1 dependent EMT activation. (Wang *et al.,* 2017) linked IL-17/TNF-α to PD-L1 in cancer cells demonstrated that inflammatory factors directly regulate PD-L1 in cancer cells. This study demonstrated that IL-6/TNF-α/PD-L1 axis operates in HBV-infected LO2 cells independently of tumor context.

Compared to recent advances in HBV therapeutics, our findings reveal DHA's unique multi-target action. Versusanoparticle vaccines, while (Wang *et al.,* 2020) developed an iron oxide nanoparticle vaccine targeting lymph node SIGNR1$^+$ cells to induce anti-preS1 antibodies (achieving functional cure in Adeno associated virus-HBV mice). DHA operates via direct modulation of the hepatic inflammation-oncogenic cascade, offering a new orally bioavailable alternative to injectable biologics. Moreover, unlike PD-L1 checkpoint inhibitors (e.g., atezolizumab, block the binding of PD-L1 to the PD-1/B7.1 receptor and activate T cells to kill tumor cells) that systemically block T-cell exhaustion but risk immune-related hepatotoxicity, DHA downregulates PD-L1 at the transcriptional level without inducing widespread immune activation, aligning with strategies to normalize the hepatic microenvironment.

Even though China has HBV vaccination program, protection wanes after 7 to 9 years in adults, and about 5% fail seroconversion due to genetic or metabolic factors (Lu *et al.,* 2021). For vaccine non-responders (e.g., smokers or hepatitis C co-infected patients with reduced anti-HBs titers), DHA might lower baseline inflammation, potentially improving booster response (Daryani *et al.,* 2007). Therefore, in high-risk groups with IL-6$^+$/EMT$^+$ profiles, DHA may hold paramount importance in delaying the progression of liver cirrhosis. Although HBV infected LO2 cells model provides evidence for HBV's EMT induction, the absence of immune components precludes evaluation of PD-L1's immunomodulatory functions. Future studies should explore synergies with checkpoint inhibitors given PD-L1's dual role in immune

evasion and EMT. Moreover, co-administering DHA with PD-L1 inhibitors may synergistically restore immune surveillance while suppressing EMT. Whether DHA can reverse EMT enhances T-cell infiltration (as in other cancer models) also warrants investigation in HBV-infected liver organoids. With the application of pharmacology, bioinformatics, and structural biology techniques in vaccine development (Ahmad *et al.,* 2024), specifically screen the binding sites of DHA against hepatitis B inflammation may lead to develop new drugs that enhance the seroconversion of vaccines.

Conclusion

In summary, this study elucidates the dualistic impact of HBV on LO2 cells, revealing a critical pathway and therapeutic intervention point. It demonstrates that HBV infection suppresses proliferation (reduced cell multiplication, decreased G1 phase cells and increased G2/M arrest) while simultaneously promoting migration and invasion (enhanced wound closure, elevated invasion). Mechanistically, HBV activates an IL-6/TNF-α/PD-L1 signaling axis, driving these phenotypes by inducing EMT (down-regulated E-cadherin/α-catenin, up-regulated N-cadherin) and inhibiting apoptosis (reduced bax/bcl-2 ratio). Crucially, we provide novel evidence that HBV can directly initiate EMT in non-cancerous hepatocytes, positioning PD-L1 as a pivotal link connecting inflammation to early pro-malignant processes. DHA powerfully counteracts HBV's oncogenic drive: it reverses HBV-induced proliferation suppression (normalized growth kinetics/clonogenicity) and abolishes HBV-driven migration/invasion, concomitant with restoring EMT and apoptosis marker expression. This establishes DHA's multi-target efficacy-simultaneously targeting viral inflammation, reversing established pre-malignant phenotypes (EMT, migration), and restoring cell cycle control. These findings underscore the scientific value of defining PD-L1's role in HBV-mediated hepatocyte transformation and offer significant applicability: DHA emerges as a compelling repurposing candidate for preventing HBV-related disease progression, particularly in patients with elevated IL-6. While this in vitro model validates DHA’s anti-HBV mechanism, future work should address pharmacokinetic optimization in hepatic tissue and evaluate efficacy in in vivo models (e.g., HBV-infected mice) to advance clinical translation.

Acknowledgement

We would like to thank AJE for the English language editing services.

Funding information

The present study was supported by the Natural Science Foundation of Chongzuo (Grant No. chongkegong 2023ZC0306) and (Grant No. chongke 20220627).

Author’s contribution

Yingde Nong and Caifang Ma: contributed equally to this study: performed the experiment and data analyses and wrote the manuscript.
Yichong Ning and Wei Huang: performed the conceptualization and manuscript preparation.
Shanbin Chen, Xueqing Guo, Huihua Deng and Yongqiang Wei: performed the research and investigation process.
Wenmei Zhao: performed the reviewing and editing.

Ethics

All authors have reviewed the final manuscript and provided explicit consent for its publication. There are not any ethical issues to declare that could arise after the publication of this manuscript.

References

Ahmad, A., Karim, A., Arfah, R. A., Agus, R., Ladju, R. B., Hidayah, N., Irfandi, R. (2024). Expression and Epitope Prediction of the Sirohydrochlorin Cobaltochelatase Isolated from a Local Strain of Mycobacterium Tuberculosis. Emerging Science Journal, 8(4), 1345-1365. doi:10.28991/ESJ-2024-08-04-07

Bekçibaşı, M., Deveci, Ö., Oğuz, A., Bozkurt, F., Dayan, S., & Çelen, M. K. (2021). Serum TNF-α, IL-1β, and IL-6 levels in chronic HBV-infected patients. Int J Clin Pract, 75(8), e14292. doi:10.1111/ijcp.14292

Chan, L, C., Li. C. W., Xia, W., Hsu, J. M., & Hung, M. C. (2019). IL-6/JAK1 pathway drives PD-L1 Y112 phosphorylation to promote cancer immune evasion. Journal of Clinical Investigation, 129(8): 3324-3338. doi: 10.1172/JCI126022

Dai, X., Chen, W., Qiao, Y., Chen, X., Chen, Y., Zhang, K., & Zhao, J. (2024). Dihydroartemisinin inhibits the development of colorectal cancer by GSK-3β/TCF7/MMP9 pathway and synergies with capecitabine. Cancer Letters, 582(000), 15. doi: 10.1016/j.canlet.2023.216596

Dai, X., Zhang, X., Chen, W., Chen, Y., Zhang, Q., Mo, S., & Lu, J. (2021) Dihydroartemisinin: A Potential Natural Anticancer Drug. International journal of biological sciences, 17(2), 603-622. doi:10.7150/ijbs.50364

Daryani, N. E., Nassiri-Toosi, M., Rashidi, A., & Khodarahmi, I. (2007). Immunogenicity of recombinant hepatitis B virus vaccine in patients with and without chronic hepatitis C virus infection: a case-control study. World Journal of Gastroenterology, 13(2), 294-298. doi: 10.3748/wjg.v13.i2.294

Huang, L., Ma, J., & Cui, M. (2021). Circular RNA hsa_circ_0001598 promotes programmed death-ligand-1-mediated immune escape and trastuzumab resistance via sponging miR-1184 in breast cancer cells. Immunologic research, 69(6), 558-567. doi: 10.1007/s12026-021-09237-w

Lan, C., Kitano, Y., Yamashita, Y. I., Yamao, T., Kajiyama, K., Yoshizumi, T., . . . Baba, H. (2022). Correction: Cancer-associated fibroblast senescence and its relation with tumour-infiltrating lymphocytes and PD-L1 expressions in intrahepatic cholangiocarcinoma. Br J Cancer, 126(2), 311-312. doi:10.1038/s41416-021-01615-3

Lu Y Y, Liang C F, Lin J P, Lu H, Wang H N, & Li H. (2021). Research Progress on Immune Response Effect of Hepatitis B Vaccine. Popular science & technology (008), 023.

Meng, J., Peng, J., Feng, J., Maurer, J., Li, X., Li, Y., . . . Kong, B. (2021). Niraparib exhibits a synergistic anti-tumor effect with PD-L1 blockade by inducing an immune response in ovarian cancer. J Transl Med, 19(1), 415. doi:10.1186/s12967-021-03073-0

Nobel, S. M. N., Sifat, O. F., Islam, M. R., Sayeed, M. S., & Amiruzzaman, M. (2024). Enhancing GI Cancer Radiation Therapy: Advanced Organ Segmentation with ResECA-U-Net Model. Emerging Science Journal, 8(3), 999-1015. doi:10.28991/ESJ-2024-08-03-012

Sitio, R., Akmal, M., Marlina, M., & Gholib, G. (2024). Investigating Ethanolic Extract from Acehnese Lime (Citrus aurantifolia) Peel as Potential Anti-Hypercholesterolemia Agent. Journal of Human, Earth, and Future, 5(3), 348-365. doi:10.28991/HEF-2024-05-03-04

Tao P Y. (2016). The preliminary study effect of combination of dihydroartemisnin and doxorubicin on multidrug resistance reversal on HCT8/ADR colon cancer cells. Guangzhou University of Chinese Medicine.

Ting, X., Qiang, Z., Shumin, Z., Wei-Long, Z., Yuan, Q., Zhun, B., Bi-Jiao, Z. (2018). Protease-activated receptor-1 (PAR1) promotes epithelial-endothelial transition through Twist1 in hepatocellular carcinoma. Journal of Experimental & Clinical Cancer Research Cr, 37(1), 185. doi: 10.1186/s13046-018-0858-4

Wang, W., Zhou, X., Bian, Y., Wang, S., & Zhu, M. (2020). Dual-targeting nanoparticle vaccine elicits a therapeutic antibody response against chronic hepatitis B. Nature Nanotechnology, 5(5): 406-416. doi: 10.1038/s41565-020-0648-y

Wang, X., Yang, L., Huang, F., Zhang, Q., Liu, S., Ma, L., & You, Z. (2017). Inflammatory cytokines IL-17 and TNF-α up-regulate PD-L1 expression in human prostate and colon cancer cells. Immunology Letters, 184: 7-14. doi: 10.1016/j.imlet.2017.02.006

Wu, D., Chen, W., Yang, Y., Qin, Y., Zu, G., Zhang, Y., Chen, X. (2023). PITX2 in pancreatic stellate cells promotes EMT in pancreatic cancer cells via the Wnt/β-catenin pathway. Acta Biochimica et Biophysica Sinica, 55(9), 11. doi: 10.1016/j.imlet.2017.02.006

Yang, X., & Liu, J. (2021). Targeting PD-L1 (Programmed death-ligand 1) and inhibiting the expression of IGF2BP2 (Insulin-like growth factor 2 mRNA-binding protein 2) affect the proliferation and apoptosis of hypopharyngeal carcinoma cells. Bioengineered, 12(1), 7755-7764. doi:10.1080/21655979.2021.1983278.

Yuen, M. F., Chen, D. S., Dusheiko, G. M., Janssen, H. L. A., Lau, D. T. Y., Locarnini, S. A., . . . Lai, C. L. (2018). Hepatitis B virus infection. Nat Rev Dis Primers, 4, 18035. doi:10.1038/nrdp.2018.35

Yu W Y, Kan W J, Yu P X, Li M M, Song J S, & Zhao F. (2013). Anti-inflammatory effect and mechanism of artemisinin and dihydroartemisinin. China Journal of Chinese Materia Medica. 37(17): 2618-2622.

Zahara, E., Darmawi, Balqis, U., & Soraya, C. (2024). The Potential of Ethanol Extract of Aleurites Moluccanus Leaves as TNF-α Inhibitor in Oral Incision Wound Care Model. Journal of Human, Earth, and Future, 5(4), 674-687. doi:10.28991/HEF-2024-05-04-010.

Zhang R, Yang B, Li H Z, Hua J, Zhou Y T, Zhou F J, & Zhang T J. (2021). Research Progress on the Anti-inflammatory Mechanism of Dihydroartemisinin. China Pharmacy, 32(9), 6.

Appendix S1. Nomenclature

Abbreviation	Reference Standard
DHA	Dihydroartemisinin
LO2	Immortalized human normal hepatocyte cell line
HBV	Hepatitis B virus
HBeAg	Hepatitis B e-antigen
HBsAg	Hepatitis B Surface Antigen
Anti-HBs	Hepatitis B surface antibody
NF-κB	Nuclear factor κ-B
IL-6	Interleukin-6
PEIU	Paul-Ehrlich-Institute
MTT	Thiazolyl Blue
TNF-α	Tumor necrosis factor-α
PD-L1	Programmed death ligand-1
Bcl-2	B-cell lymphoma-2
Bax	Bcl-2-associated X protein
P21	Cyclin dependent kinase inhibitor 1A
EMT	Epithelial mesenchymal transition
WB	Western blotting