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Histidine ammonia lyase expression characteristics in primary liver cancer: hepatocellular carcinoma and cholangiocarcinoma

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Abstract

Background Histidine (His) ammonia Lyase (HAL) is a key rate-limiting enzyme that catalyzes the first reaction in the metabolism of histidine. Deficiencies in HAL lead to histidinemia, characterized by elevated levels of His in the blood and other body fluids. The level of HAL in these fluids is known to impact the sensitivity of cancer cells to certain chemotherapeutics by regulating His. However, the molecular role and impacts of HAL in the progression of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are unknown at present. Thus, we investigated the expression of HAL in HCC and CCA tumors.

Method The Cancer Genome Atlas (TCGA) database, quantitative real-time PCR (qRT-PCR), and immunoblot analysis were utilized to examine the expression of HAL in HCC and CCA patients.

Results TCGA analysis revealed a significant downregulation of HAL mRNA expression in HCC and CCA tumor tissues compared with normal tissues ($p < 0.0001$). Our qRT-PCR and immunoblot analysis demonstrated a significant decrease in both HAL mRNA (HCC, $n = 9$, $p < 0.05$; CCA, $n = 7$, $p < 0.05$) and protein levels (HCC, 91.7%, 22/24; CCA, 92.9%, 13/14) in both HCC and CCA tumor tissues compared with adjacent non-tumor liver/ bile duct tissues across the majority of patient samples analyzed. The protein expression pattern aligns with the mRNA findings, indicating that HAL downregulation occurs at both transcriptional and protein levels.

Conclusion Our data evinced the relevance and significance of HAL in the tumorigenesis of HCC and CCA. These findings suggest that HAL and its metabolic derivatives can be developed as a potential prognostic and diagnostic biomarker as well as therapeutics for HCC and CCA.

Keywords HCC, CCA, HCV, HAL

1 Introduction

Primary liver cancer is the sixth most common cancer worldwide and the third leading cause of cancer-related deaths [1]. It comprises a diverse group of malignancies characterized by distinct risk factors, clinical outcomes, as well as histological and molecular features. Among these malignancies, HCC and CCA, the two most commonly reported primary liver cancers, account for 80–90% and 10–15% of the cases, respectively [2–4]. HCC, the most prevalent form of primary liver cancer, results from the malignant transformation of hepatocytes, typically occurring in the context of chronic liver disease or cirrhosis [4–6]. In the United States, HCC incidence has been rising due to the increasing prevalence of chronic hepatitis C infection, non-alcoholic fatty liver disease, and non-alcoholic steatohepatitis, which are associated with obesity and metabolic syndrome [4]. CCA, the second most common primary hepatic malignancy, is an aggressive cancer that originates from the biliary epithelium, both inside and outside the liver. Despite advancements in the diagnosis and treatment of these primary liver cancers, the five-year survival rates in the USA remain dismal, with less than 20% for HCC and 10% for CCA, respectively [2, 7].

The management of liver cancer, particularly HCC and CCA, is challenged by the heterogeneity of the disease, inadequate markers for early diagnosis, the lack of specific markers to identify the tumor stage, and the development of drug resistance [2, 8–10]. Due to these critical gaps, there is an ongoing quest to better understand the role of various genes and proteins involved in the development and progression of HCC and CCA in humans. These research endeavors aim to evaluate the potential clinical value of these signature molecules as biomarkers for diagnosis, prognosis, and clinical management [11–13].

Histidine Ammonia Lyase (HAL), also known as histidase or histidinase, is a cytosolic enzyme primarily found in the liver and skin. It catalyzes the first reaction in the catabolism of histidine, which involves the irreversible non-oxidative deamination of L-histidine to trans-urocanic acid and ammonia [14–17]. Proper histidine metabolism is necessary to prevent the accumulation of histidine and its related metabolic disorders. It also plays a critical role in synthesizing purines and tetrahydrofolate, as well as maintaining amino acid homeostasis [18]. Furthermore, histamine, a metabolite of histidine, influences both innate and adaptive immune responses [14, 15]. Decreased activity of HAL has been linked to histidinemia or histidiuria, which is associated with developmental challenges in children [16, 19]. HAL has been studied in various contexts, including skin pigmentation [20], milk production in cattle [21], and several types of cancer [15, 22–25].

In medulloblastoma, HAL has been studied as a source of neo-antigens that can be targeted by immunotherapies for pediatric medulloblastoma [23]. Depending on the type of cancer, HAL's expression levels vary and appear contradictory. Leukemia patients with high HAL expression levels had higher survival rates than those with low HAL expression levels. Additionally, HAL expression was significantly higher in hematopoietic cell lines that were identified as highly sensitive to methotrexate. In contrast, those with low HAL expression showed low sensitivity to methotrexate. Genetic depletion of HAL decreased sensitivity to methotrexate in acute lymphoblastic leukemia cell lines [24]. This suggests that HAL plays a significant role in enhancing the efficacy of methotrexate treatment in leukemia and could potentially serve as a biomarker for predicting

patient response and overall prognosis. In pancreatic cancer, HAL has been reported to be upregulated. Analysis of The Cancer Genome Atlas (TCGA) database, along with studies using pancreatic cancer patient tissues and cell lines, revealed significant upregulation of HAL mRNA and protein expression compared to normal tissues or the pancreatic cell lines used. Immunohistochemical analysis further confirmed increased HAL expression in human pancreatic cancer tissues and pancreatic sections from mice, suggesting a potential role for HAL in the development of pancreatic cancer [15].

However, in diethyl nitrosamine-induced hepatoma, HAL's catalytic activity and protein expression were diminished in rats [25], suggesting that the downregulation of HAL may play a role in the development and progression of liver cancer induced by diethyl nitrosamine. Additionally, previous microarray data revealed HAL as one of the key genes downregulated in a stage-specific manner in liver disease by HCV infection [26, 27]. Although the expression of HAL is critical for hepatocarcinogenesis, little studies are available on HAL's role in HCC and CCA. The role of HAL expression in HCC and CCA patient samples remains to be explored. Taken together, these reports indicate that the degree of contribution of HAL to the progression of disparate tumors differs; thus, establishing the relevance of the level of HAL to the progression of specific tumors is imperative for the development of prognostic and diagnostic biomarkers and therapeutics. Hence, in this study, we investigate the expression of HAL in HCC and CCA progression using patient tissue samples and their matching adjacent non-tumor tissues.

2 Materials and methods

2.1 Cell lines

Human hepatoma-derived Huh7.5.1 cell line and its subgenomic replicon, APC140, which expresses all regulatory proteins essential for HCV replication as well as the structural Core protein of the JFH1 strain of HCV, were obtained from Apath, L.L.C. These cells were cultured in DMEM (Corning) supplemented with 10% fetal bovine serum (GIBCO), according to the manufacturer's protocol. The APC140 cells were cultured in the presence of 0.5 g/L geneticin. Both cell lines were maintained in a humidified incubator at 37 °C with 5% CO_2.

2.2 Analysis of mRNA expression of HAL in pan-cancer

The mRNA expression data for HAL across 23 cancer types was downloaded from The Cancer Genome Atlas (TCGA) database using the University of California, Santa Cruz (UCSC) browser [28]. The data comprised tumor and normal tissues, and sample sizes varied across the cancer types. RNA-Seq data were preprocessed using normalized RNA-Seq by Expectation Maximization (RSEM) values, with an offset of + 1 to avoid log transformation issues with zero values. The data were then Log2 transformed for analysis. Box plots were generated using the ggplot2 package in R (version 4.4.1), displaying the median, interquartile range (IQR), and whiskers extending to 1.5× IQR.

2.3 HCC tumor tissue sample collection

Deidentified frozen tumor tissues from HCC and CCA, along with matching adjacent non-tumor tissue samples (HCC: $n = 24$, CCA: $n = 14$), were provided by the Ontario Tumour Bank in Toronto, Canada, and the Alberta Cancer Research Biobank in Calgary, Canada. The samples were stored at -80 °C and thawed on ice prior to use. All archived

and deidentified tumor tissue samples were obtained under Institutional Review Board (IRB) protocols approved by the University of North Texas Health Science Center in Fort Worth, TX, USA.

2.4 RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA from the cells was isolated using TRIzol reagent (Invitrogen) and from the frozen tissues using the RNeasy Mini Kit (Qiagen), according to the instructions provided by the manufacturer. The total RNA was quantified with a NanoDrop 2000 instrument (Thermo Scientific) and complementary DNA (cDNA) was synthesized using TaqMan Reverse Transcription Reagents (Applied Biosystems). The mRNA expression levels were determined by qRT-PCR with THUNDERBIRD Next SYBR qPCR Mix (Toyobo, Osaka, Japan) and QuantStudio 3 Real-Time PCR System (Applied Biosystems) using gene-specific primers for HAL (sense primer: 5′-CCACTAAGTCCTGAGAGGT GTC-3′ and antisense primer: 5′-CACCAACGGTTCCTTTCTCTGG-3′), and GAPDH (sense primer: 5′-GTCTCCTCTGACTTCAACAGCG-3′ and antisense primer: 5′-ACC ACCCTGTTGCTGTAGCCAA-3′). GAPDH was used as an endogenous control to normalize the data. The average of the ΔCt values of all the normal samples was used to obtain the ΔΔCt values for each individual sample (non-tumor liver tissue and HCC). This ΔΔCt was then used to determine the fold change (the normalized expression values).

2.5 Preparation of tissue extracts and Immunoblot analysis

Frozen patient tissues were thawed and lysed using RIPA buffer supplemented with protease and phosphatase inhibitor, and sonicated (Pulse 150, ultrasonic homogenizer) for thorough cell disruption. Protein concentrations were measured using the BCA assay to ensure equal loading. Proteins were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. Blocking the membrane with 5% non-fat milk was then followed by incubation with the specific primary antibody at 4°C overnight on a shaker. After washing the membrane in Tris Buffered Saline with Tween 20 three times for 10 min each, it was then incubated with the required horseradish-peroxidase–conjugated secondary antibody. The target protein bands were visualized using the chemiluminescence detection method (ProteinSimple Alpha Innotech FluorChem Q). Densitometry analysis was carried out using Image J software to quantify and analyze the band intensities. Specifically, a uniform rectangle was drawn around each band, and background subtraction was applied to accurately determine the total pixel density for each lane. The ratio of HAL expression relative to loading control was calculated, and the graphical representation of the quantified data was generated using GraphPad Prism 8 software. The primary antibodies used for immunoblotting were as follows: anti-HAL (ABclonal Technology #A13021 and Proteintech #25940-1-AP), anti-Hepatitis C NS3 (ViroGen #217-A), anti-HCV NS5A (ViroGen #276-A), anti-NS5B (Abcam #ab65410), and anti-GAPDH (Santa Cruz Biotechnology #sc-32233).

2.6 Statistical analyses

GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses, and results were presented as mean ± SEM. All comparisons were made based on the control using a two-tailed Student's *t*-test and considered significant if the *p*-value was at least ≤ 0.05: (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$; (****) $p < 0.0001$.

3 Results

3.1 Expression of HAL in stage-specific advanced HCV liver disease

Our previous Affymetrix Human Transcriptome Array (HTA2.0) analysis identified 75 genes that are differentially expressed in liver samples from early versus advanced HCV-infected patients [26, 27]. We found that the expression of the gene HAL is significantly downregulated in patients with advanced HCV infection compared to those with early-stage infection. Given HAL's known functions, we further explored its expression in pan-cancer datasets from The Cancer Genome Atlas (TCGA), as well as in HCC tumor tissues and HCV-expressing APC140 cell line. This investigation aimed to assess the potential of developing HAL as a biomarker and therapeutic target in the progression of HCV-mediated hepatocellular carcinoma (HCC).

3.2 The mRNA expression of HAL across various cancers

The mRNA expression of HAL in tumor tissues and adjacent non-tumor tissues was visualized using box plots created from 23 selected cancer types in the pan-cancer dataset from The Cancer Genome Atlas (TCGA) (Fig. 1). These plots provide a comparative overview of HAL expression levels between normal and tumor tissues across these cancer types, highlighting noticeable variations. The findings indicate that HAL mRNA expression is significantly upregulated in kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD), and uterine endometrial carcinoma (UCEC). Conversely, it is downregulated in cholangiocarcinoma (CCA), kidney chromophobe (KICH), hepatocellular carcinoma (HCC), lung squamous cell carcinoma (LUSC), thyroid carcinoma

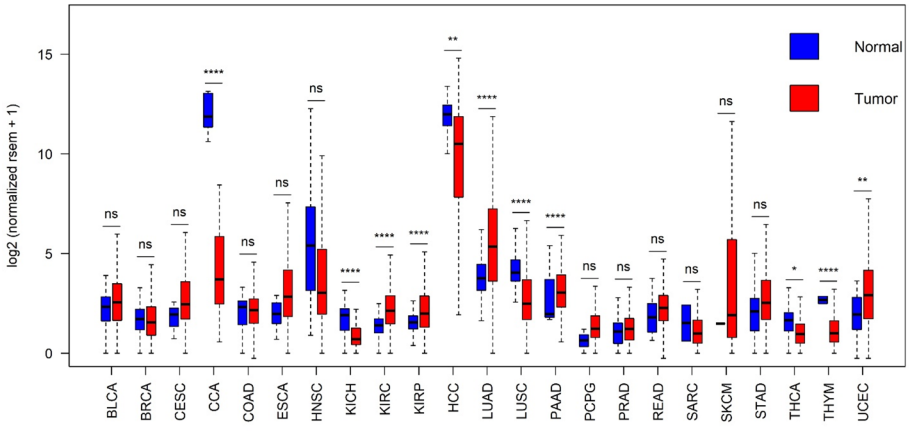

Fig. 1 The mRNA expression of HAL across various cancer types from TCGA database: Box plot showing the differential mRNA expression levels of HAL across cancer tissues (red) and their normal tissues (blue) in 23 different cancer types from TCGA database. Each point represents an individual sample. The central line in each box represents the median, the edges of the box represent the interquartile range (IQR), and the whiskers extend to 1.5× IQR. Statistical analyses were performed using the two-tailed Student's t-test (ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$)

(THCA), and thymoma (THYM). No significant differences in HAL expression were observed for bladder carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), and stomach adenocarcinoma (STAD). Interestingly, HAL expression is notably higher in non-tumorous tissues of the liver and bile duct compared to other organs. However, its expression is significantly lower in HCC ($p < 0.01$) and CCA ($p < 0.0001$) tissues. This suggests that HAL plays a critical role in the diagnosis and treatment of HCC and CCA.

3.3 mRNA expression levels of HAL in HCC and CCA patients based on the TCGA database

To investigate the role of HAL in HCC, we further analyzed the expression of HAL mRNA in adjacent non-tumor liver tissues ($n = 50$) and HCC tumor tissues ($n = 371$) using TCGA database. The mRNA expression of HAL was significantly downregulated in HCC tumor tissues (mean ± SEM: 9.35 ± 0.17) compared to normal liver tissues (mean ± SEM: 11.83 ± 0.13) ($p < 0.001$, Fig. 2A). Similarly, the amount of the intracellular HAL mRNA was significantly lower in HCC tumor tissues (mean ± SEM: 9.17 ± 0.51) compared to their matching adjacent non-tumor tissue (mean ± SEM: 11.83 ± 0.13) ($p < 0.001$, Fig. 2B - C). These observations suggest that diminished expression of HAL contributes to the development or progression of HCC. The consistency observed in the matching pairs corroborates the association and suggests that the decreased HAL mRNA expression is directly linked to the tumor formation rather than due to individual variability.

Similarly, in CCA, we analyzed the level of HAL mRNA in normal ($n = 9$) and CCA tumor tissues ($n = 36$) using TCGA database. The mRNA expression of HAL was significantly downregulated in CCA tumor tissues (mean ± SEM: 4.31 ± 0.43) compared to normal tissues (mean ± SEM: 12.09 ± 0.31) ($p < 0.0001$, Fig. 2D). HAL mRNA expression was significantly lower in CCA tumor tissues (mean ± SEM: 4.64 ± 0.85) compared to their matching adjacent non-tumor tissue (mean ± SEM: 12.09 ± 0.31) ($p < 0.0001$, Fig. 2E - F). These observations suggest that reduced expression of HAL in CCA may contribute to its development or progression. The consistency observed in the matching pairs supports the association, suggesting that the lower HAL mRNA expression is more likely related to the tumor formation than individual differences.

3.4 HAL mRNA expression in HCC and CCA patient tumor tissues

To validate and further investigate the differential expression of HAL observed from the TCGA RNAseq data sets, we performed mRNA expression analysis in paired HCC ($n = 9$) and CCA ($n = 7$) patient samples using qRT-PCR (Fig. 3). The mRNA analysis on these paired sets showed significant downregulation of HAL in the HCC tumor tissues (mean ± SEM: 0.34 ± 0.14) compared to the adjacent normal tissues (mean ± SEM: 1.22 ± 0.32) ($p < 0.05$, Fig. 3A, B). Similarly, in CCA patient samples ($n = 7$), the mRNA analysis showed significant downregulation of HAL in the CCA tumor tissues (mean ± SEM: 0.33 ± 0.18) compared with the matching adjacent normal tissues (mean ± SEM: 1.52 ± 0.42) ($p < 0.05$, Fig. 3C, $p < 0.01$, Fig. 3D: paired plot). This trend of

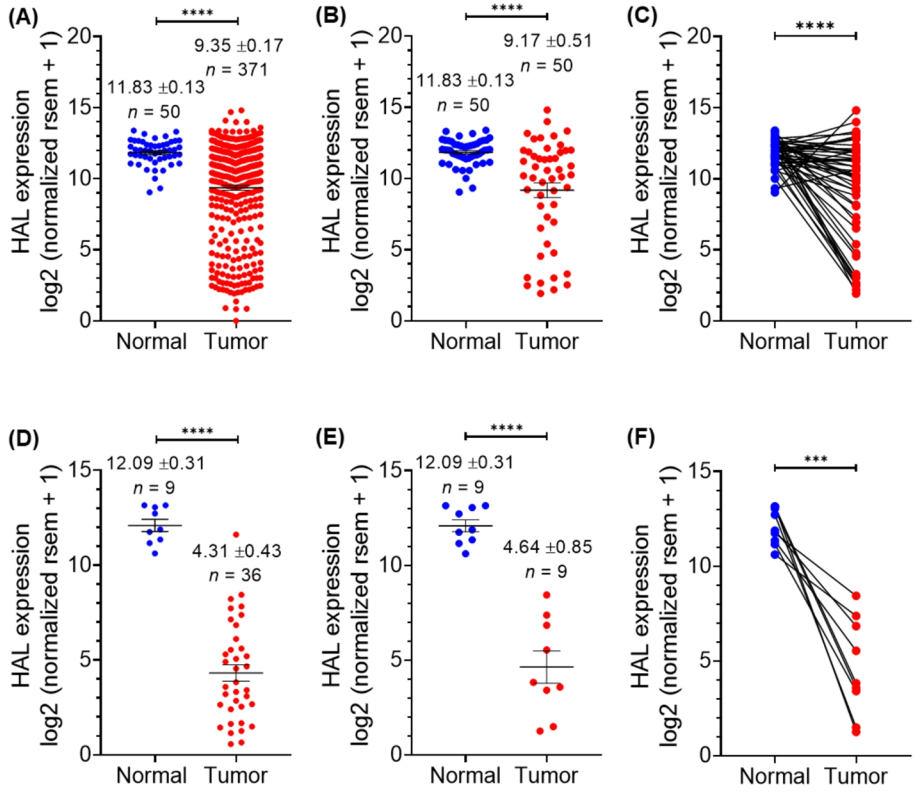

Fig. 2 mRNA expression levels of HAL in HCC and CCA patients based on the TCGA database: **A** Scatter plot analysis showing the HAL mRNA levels in HCC tumor tissues ($n = 371$) and normal tissues ($n = 50$) according to the TCGA-HCC RNAseq database. The data are expressed as the mean ± standard error of mean (SEM). Statistical analyses were performed using the two-tailed Student's t-test (****$p < 0.0001$). **B** Scatter plot analysis showing the HAL mRNA levels in HCC tumor tissues ($n = 50$) and matched adjacent non-tumor liver tissue samples ($n = 50$) obtained from TCGA-HCC RNAseq database. The data are expressed as the mean ± SEM (****$p < 0.0001$; two-tailed Student's *t*-test). **C** Paired plot analysis of HAL expression in 50 sets of HCC paired samples from TCGA database. Paired t-test was used to compare the expression of the HAL between the tumor and adjacent tissues (****$p < 0.0001$; two-tailed Student's *t*-test). **D** Scatter plot analysis showing the HAL mRNA levels in CCA tumor tissues ($n = 36$) and normal tissues ($n = 9$) according to TCGA-CCA RNAseq database. The data are expressed as the mean ± SEM (****$p < 0.0001$; two-tailed Student's *t*-test). **E** Scatter plot analysis showing the HAL mRNA levels in CCA tumor tissues ($n = 9$) and matched adjacent non-tumor tissue samples ($n = 9$) obtained from the TCGA-CCA RNAseq database. The data are expressed as the mean ± SEM (****$p < 0.0001$; two-tailed Student's *t*-test). **F** Paired plot analysis of HAL expression in 9 sets of CCA paired samples from TCGA database. Paired t-test was used to compare the expression of the HAL between the tumor and adjacent tissues (***$p < 0.001$; two-tailed Student's *t*-test)

decreased HAL mRNA expression was observed in the majority of the paired samples, therefore supporting the findings observed from the TCGA data.

3.5 HAL protein expression in HCC and CCA patient tumor tissues

To further validate the expression pattern of HAL in HCC at the protein level, we performed an immunoblot analysis of 24 paired sets of HCC patient samples (tumor tissues and their adjacent non-tumor tissues) (Fig. 4). The immunoblot analysis showed a consistent decrease in HAL protein expression in HCC tumors (T) compared to their matching adjacent non-tumor tissues (N), across the majority (22 out of 24, or 91.7%) of the samples analyzed (Fig. 4A, B).

Similarly, immunoblot analysis of HAL protein level was also evaluated in 14 pairs of CCA tumor tissues with adjacent non-tumor tissues (Fig. 5). The results of the immunoblot analysis consistently showed a decrease in HAL protein expression in CCA

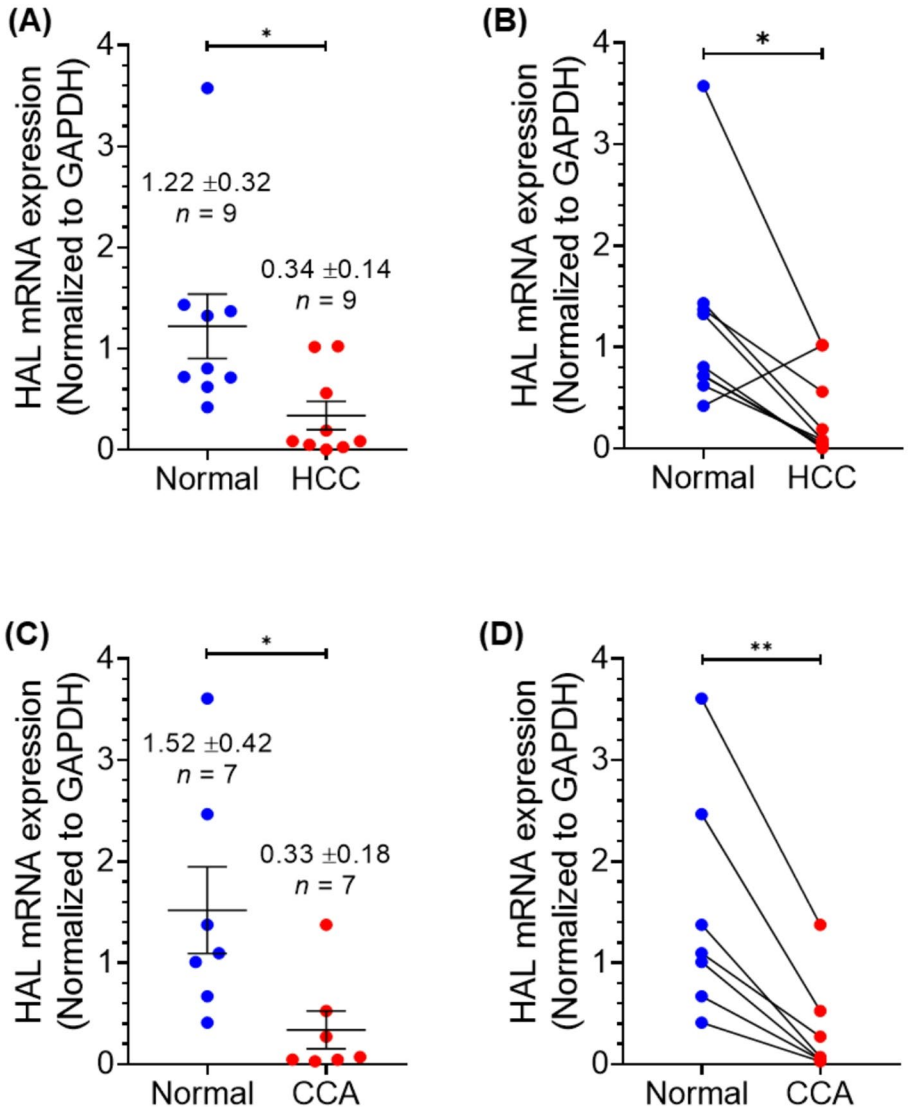

Fig. 3 HAL mRNA expression in HCC and CCA tumor tissues: **A** Scatter plot analysis of HAL mRNA fold expression in normal liver tissues ($n=9$) and HCC tumor tissue samples ($n=9$) normalized to GAPDH. The data are expressed as the mean ± SEM (*$p<0.05$; two-tailed Student's *t*-test). **B** Paired plot analysis of HAL mRNA expression in 9 pairs of HCC paired samples, normalized to GAPDH (*$p<0.05$; two-tailed Student's *t*-test). **C** Scatter plot analysis of HAL mRNA fold expression in normal bile duct tissues ($n=7$) and CCA tumor tissue samples ($n=7$), normalized to GAPDH. The data are expressed as the mean ± SEM (*$p<0.05$; two-tailed Student's t-test). **D** Paired plot analysis of HAL mRNA expression in 7 pairs of CCA paired samples, normalized to GAPDH (**$p<0.01$; two-tailed Student's t-test)

tumors (T) when compared to their corresponding adjacent non-tumor tissues (N). This trend was observed in the majority of the samples analyzed, with 13 out of 14, or 92.9%, demonstrating reduced expression (Fig. 5A, B). These findings align with the mRNA expression data, further supporting the observation that HAL is downregulated in both HCC and CCA, both at the transcriptional and translational levels.

3.6 Expression of the HAL in HCV subgenomic replicon cells

Since HCV is a major culprit for HCC and CCA, based on previous transcriptome array data and our current observations of HAL expression in liver cancer patient samples, we further investigated the significance of HAL in HCV-mediated tumorigenesis. To this

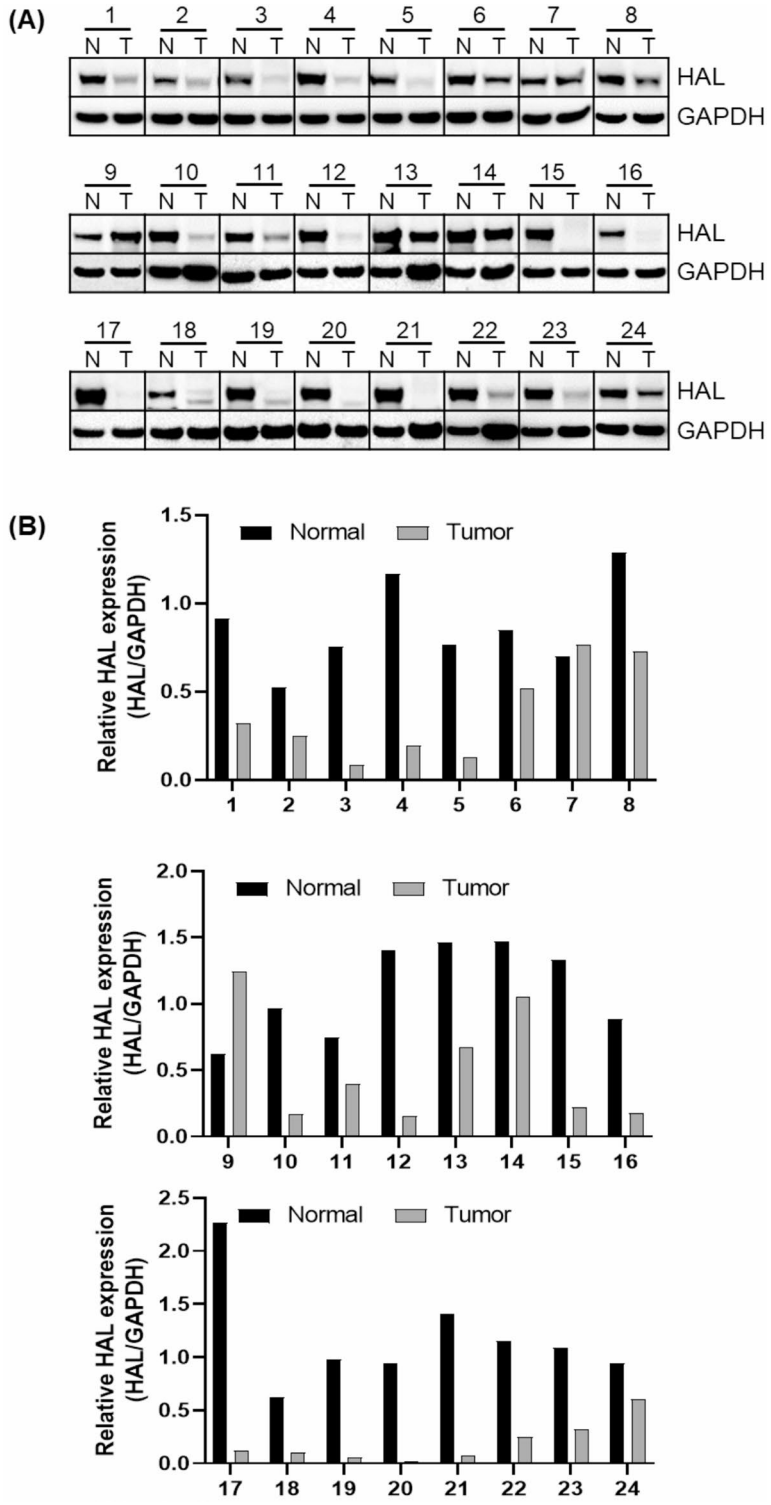

Fig. 4 HAL protein expression in HCC tumor tissues: **A** Immunoblot analysis showing the protein level of HAL in 24 pairs of clinical HCC tumor (T) samples and adjacent normal (N) liver tissue samples. GAPDH was used as a loading control. **B** Bar graph showing the densitometric analysis of HAL bands of the immunoblot of panel A. Intensity of HAL bands was normalized by GAPDH loading control. Uncropped immunoblots of (A) are available in supplementary Figure S1

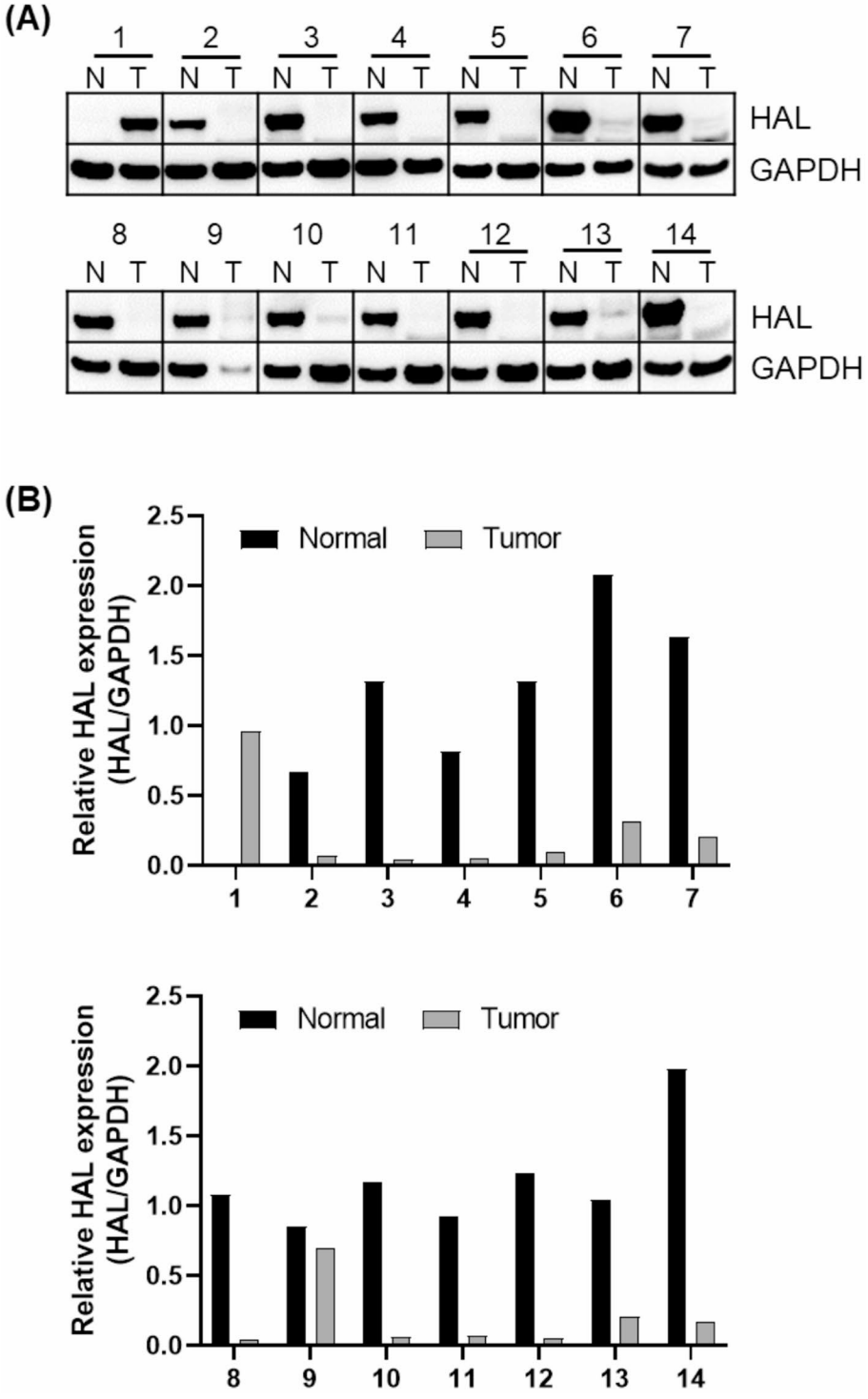

Fig. 5 HAL protein expression in CCA tumor tissues: **A** Immunoblot analysis showing the protein level of HAL in 14 pairs of clinical CCA tumor (T) samples and adjacent normal (N) tissue samples. GAPDH was used as a loading control. **B** Bar graph showing the densitometric analysis of HAL bands of the immunoblot of panel A. Intensity of HAL bands was normalized by GAPDH loading control. Uncropped immunoblots of (A) are available in supplementary Figure S2

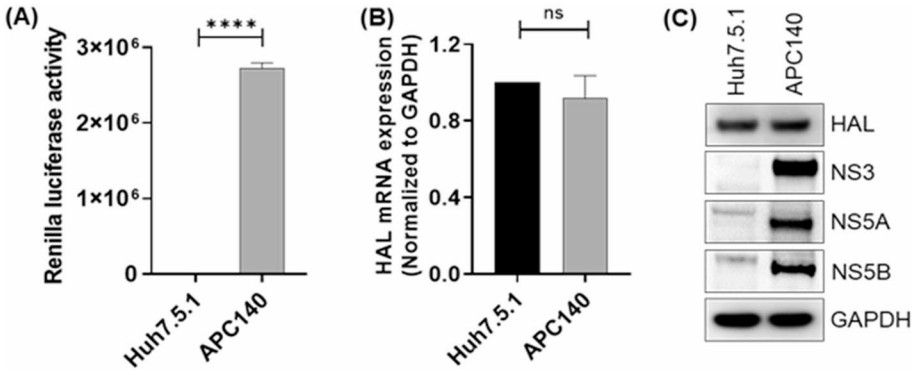

Fig. 6 HAL mRNA and protein expression in HCV-positive cells: **A** Bar graph showing the Renilla luciferase activity in Huh7.5.1 and APC140 cell lines. **B** Bar graph showing HAL mRNA expression in Huh7.5.1 and APC140 cell lines. The housekeeping gene GAPDH was used as a loading control ($^{ns}p > 0.05$, two-tailed Student *t*-test). **C** Immunoblot analysis showing the protein expression of HAL and HCV viral proteins, NS3, NS5A, and NS5B in Huh7.5.1 and APC140 cell lines. GAPDH was used as a loading control. Uncropped immunoblots of (C) are available in supplementary Figure S3

end, we utilized HCV replicon cells, APC140, which expresses all the viral genes, except the envelope, necessary for the replication of the JFH1 strain of HCV. First, we assessed HCV expression in the APC140 cells using a Renilla luciferase assay and immunoblot analysis. The Huh7.5.1 cell line, which is negative for the HCV strain, served as a control. Our findings indicate that Renilla luciferase levels were significantly higher in the APC140 cell line compared to the Huh7.5.1 cell line (Fig. 6A). The presence of HCV was further confirmed by immunoblot analysis, which demonstrated the expression of NS3, NS5A, and NS5B viral proteins in the APC140 cell line (Fig. 6C). We performed qRT-PCR analysis to evaluate the effects of HCV on the mRNA and protein expression of HAL in both the Huh7.5.1 and APC140 cell lines. The results indicated that there were no changes in the level of HAL at the mRNA and protein levels in the HCV-positive APC140 cells compared with the HCV-negative Huh7.5.1 cells (Fig. 6B and C). This finding contradicts the previous transcriptome array data [26].

4 Discussion

Understanding the biology of the onset and progression of primary liver cancer, discovering sensitive and reliable biomarkers for early diagnosis, and developing novel therapies represent effective strategies to address the challenges associated with its management [29–32]. As part of this quest, it is essential to explore the genes involved in the development of HCC and CCA to better understand the molecular mechanisms driving their pathogenesis. Research into histidine metabolism is gaining importance in cancer therapy, highlighting its therapeutic potential, physiological significance, and impact on anti-cancer drug sensitivity. Previous microarray data reported a stage-specific down-regulation of HAL, of liver disease caused by HCV, suggesting its role in liver pathology progression. This study investigated the expression of HAL, a rate-limiting enzyme in histidine catabolism, in the two most reported primary liver cancers, as its expression patterns in HCC and CCA patients remained unexplored.

Pan-cancer analyses aim to uncover shared characteristics across various cancers to identify core patterns underlying cancer development and progression [33]. This analysis explored the mRNA expression profile of HAL across 23 cancer types and identified potential patterns that may indicate HAL's role in cancer progression. The box plots

generated from the TCGA database provided a comparative view and diverse expression patterns of HAL expression in tumor versus normal tissues across these multiple cancer types. In HCC and CCA, HAL mRNA expression was notably reduced in tumor tissues compared to normal tissues, suggesting a potential role for HAL downregulation in these primary liver cancers.

Further TCGA analysis revealed a significant downregulation of HAL mRNA in HCC and CCA tumor tissues compared to normal tissues. Similarly, mRNA and protein expression analyses in the patient samples studied showed lower HAL expression compared to their matched non-tumor pairs. Previous studies have suggested that HAL is differentially expressed in various cancers [15, 22–25] and may play a role in metabolic reprogramming in cancer cells. HAL expression has been reported to be high in leukemia and pancreatic cancer, in contrast with diminished expression in diethyl nitrosamine-induced hepatoma [25]. Our results aligned with the latter, demonstrating reduced expression in both HCC and CCA. Moreover, our study provides unique evidence from patient samples of HCC and CCA tissues. In contrast, high HAL expression in pancreatic cancer may promote tumor growth, while in leukemia, high levels drive metabolic reprogramming and proliferation, and low levels may indicate less aggressive disease. In medulloblastoma, elevated HAL acts as a neoantigen eliciting $CD8^+$ T-cell responses, whereas low expression may reduce immune recognition. These patterns demonstrate that HAL's level- and context-dependent expression may carry distinct functional implications.

The consistently low expression of HAL in HCC and CCA suggests that HAL may have a tumor-suppressive role, a protective function, or a metabolic regulatory role. By reducing HAL expression, HCC and CCA cells could evade HAL's regulatory role to support cell proliferation and cancer cell survival. This low HAL expression could also support a metabolic shift that facilitates tumor growth. This may be achieved by modulating histidine metabolism or altering amino acid metabolism, which could lead to increased availability of substrates necessary for cancer cell proliferation. Altered amino acid metabolism is a common feature of tumors and is linked to dysregulated signaling pathways [34]. For instance, histidine (along with serine and arginine) is transported into cells in exchange for asparagine export, which is crucial for maintaining amino acid balance and protein synthesis [26]. Low HAL levels may increase intracellular histidine levels, indirectly enhancing processes such as asparagine export and subsequent amino acid import, fueling protein production and tumor growth.

Our observation can be mechanistically linked to dysregulation of the Wnt/β-catenin signaling pathway, which is frequently activated in various cancers [35]. In liver cancer cells, previous studies have shown that the β-catenin/T-cell factor (TCF) complex transcriptionally suppresses HAL, in part by inhibiting liver-enriched transcription factors CCAAT/enhancer-binding protein alpha (CEBPA) and forkhead box protein A1 (FOXA1) [14]. These factors not only regulate HAL but also modulate other metabolic genes, including arginase 1 (ARG1), affecting arginine metabolism. Upregulation of ARG1 depletes intracellular arginine while increasing ornithine, a precursor for polyamines that support cell proliferation and tumor growth. Activation of Wnt signaling increases intracellular levels of histidine and arginine, which are key amino acids for protein synthesis, nucleotide production, and cellular proliferation. Concurrently, Wnt activation enhances lactic acid production and alters urea cycle metabolites, collectively

driving metabolic reprogramming characterized by aerobic glycolysis (the Warburg effect) and amino acid imbalance.

Histidine degradation, facilitated by high HAL expression, has been reported to enhance cancer therapy [24, 36]. The study by Kanarek et al. [24] in leukemia patients demonstrated that HAL-driven histidine degradation depletes the cellular tetrahydrofolate (THF) pool, which can synergistically boost the cytotoxic effects of methotrexate. Since THF is essential for DNA synthesis and repair by donating methyl groups for nucleotide production, low HAL expression leads to less THF depletion. This results in an abundance of THF, which supports rapid cell proliferation and tumor growth by ensuring ample nucleotide availability. In effect, patients with high HAL expression had higher survival rates compared with those with low HAL expression. Similarly, HCC and CCA patients have low HAL expressions and hence may support this phenomenon. Low HAL expression causes high THF levels in cancer cells, making them less sensitive to treatments targeting folate metabolism. This metabolic resistance allows tumor cells to survive and continue growing despite treatment. Also, the abundance of THF supports rapid cell proliferation and tumor growth.

Histamine is synthesized from histidine through the action of histidine decarboxylase. Low levels of HAL, which metabolizes histidine, can increase histidine availability, providing more substrate for histamine production. This elevated histamine level can drive inflammation, immune suppression, and tumor progression in HCC and CCA. In HCV-associated liver disease, histamine elevation exacerbates liver disease progression by enhancing immune dysregulation [37]. This connection was further supported by studies showing that H1-antihistamines reduce the risk of HCC in HCV patients [38, 39]. In CCA, histamine promotes CCA growth via the release of histamine from cholangiocytes. The released histamine stimulated the proliferation of both small and large cholangiocytes [40]. Subsequent studies revealed that histamine signaling via H4 receptors drives CCA proliferation, angiogenesis, epithelial-to-mesenchymal transition, and extracellular matrix breakdown [41]. In glioblastoma stem cells, increased histamine synthesis and secretion have been shown to enhance tumor progression by promoting angiogenesis [42]. Moreover, Zhang et al. (2024) reported that histamine-related genes contribute to an immunosuppressive tumor microenvironment in HCC [43]. Thus, the resulting increase in histamine in both HCC and CCA likely promotes a pro-tumorigenic microenvironment that drives the aggressiveness of these primary liver cancers. Overall, reduced HAL expression disrupts histidine metabolism, leading to heightened histamine-driven inflammation, angiogenesis, immune suppression, and tumor growth [42, 44, 45].

As such, the expression level of HAL plays a critical role in the tumorigenesis of HCC and CCA. Our previous data indicated that the level of HAL was remarkably reduced in the liver disease stage-specific manner in HCV-mediated HCC, indicating the significance of the gene in both virus-and non-virus-mediated HCC. However, interestingly, the changes of HAL were not observed with the APC140 replicon cells in which all essential viral proteins for virus replication, but not infectious progeny viruses, are produced. Further investigation is needed to elucidate the disparities in HAL expression in infectious progeny HCV-producing primary hepatocytes.

HAL is consistently downregulated in HCC and CCA, making it a promising diagnostic and prognostic biomarker with clinical translational potential. Its mRNA or protein

expression can be quantified in liver tissue via qRT-PCR, or immunohistochemical assays. HAL may complement AFP or Des-gamma-carboxy prothrombin (DCP), particularly in AFP-negative patients, and improve stratification by tumor differentiation and metabolic status. HAL reflects hepatic amino acid metabolism and differentiation pathways; hence, longitudinal monitoring could indicate tumor metabolic shifts, therapeutic response, or early recurrence. For example, a further decline in HAL levels over time might suggest disease advancement or resistance, whereas stabilization or restoration of HAL expression could correlate with treatment response or remission. Integrating HAL into biomarker panels may enable more precise prognosis and personalized management.

While our results provide some level of analysis of HAL expression in HCC and CCA, this study is limited by the small sample size of patient tissues. This may also not cover the full heterogeneity of HCC and CCA. Investigating HAL expression across a larger, more diverse cohort can validate its use as a reliable biomarker. Additionally, further in vitro and in vivo studies, particularly those involving knockdown and over-expression, could elucidate the mechanistic role of HAL in HCC and CCA.

5 Conclusion

The consistent downregulation of HAL observed across TCGA data, as well as in patient samples analyzed through qRT-PCR and immunoblotting, provides compelling preliminary evidence that the reduced expression of HAL contributes to the development and progression of HCC and CCA. These findings indicate that HAL could serve as a potential biomarker for HCC or CCA, underscoring the need for further research into therapeutic strategies aimed at restoring HAL expression to inhibit tumor growth.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s12672-025-04043-4.

Supplementary Material 1.

Author contributions

HKF, TH, and PC conceived, designed, and performed experiments and interpreted data. HKF, IWP, HPJ, and PC wrote the manuscript. IWP and PC were responsible for the funding.

Funding

This research was partly supported by the National Institutes of Health grant CA283524 (PC), AI179337 (IWP) and the UNTHSC Seed Grant to PC and IWP. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data availability

The RNA-seq datasets used in this study were obtained from the TCGA database through the UCSC Xena platform (https://xenabrowser.net/). Additionally, uncropped immunoblots for Figures 4A, 5A, and 6C are provided in the supplementary material.

Declarations

Ethics approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the University of North Texas Health Science Center, Fort Worth, TX, USA (IRB approval number: 2023-034).

Consent to participate

Patient consent was waived due to the usage of deidentified and existing tumor tissues which were collected from two biorepository centers: (1) Ontario Tumour Bank in Toronto, Canada, and (2) the Alberta Cancer Research Biobank in Calgary, Canada. According to the NIH guidelines for Protection of Human Research Subjects (45 CFR 46) and our IRB protocol, this study is exempted from the Human Subjects research.

Consent for publication
All authors have read and approved the final manuscript and consent to its publication.

Competing interests
The authors declare no competing interests.

Received: 14 August 2025 / Accepted: 6 November 2025
Published online: 28 November 2025

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