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Review Article

Acetaldehyde Dehydrogenases in Liver Zonation and Liver Cancer

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Abstract

The liver maintains important homeostatic functions such as metabolism and detoxification. Failure to remove toxic intermediates can cause hepatic damage, liver fibrosis, and even cancer development. This review focuses on acetaldehyde dehydrogenases (ALDHs), a group of key enzymes within the ALDH superfamily with the ability to convert highly reactive aldehyde substrates to the corresponding carboxylic acids in NAD(P)-dependent manners. These enzymes participate in a diverse array of biological processes such as detoxification, biosynthesis, antioxidant, and regulatory functions. ALDH dysfunction can disrupt homeostasis, leading to toxic buildup, tissue damage, and cancer. Here, we examine the expression patterns of hepatic ALDHs in adult normal human livers and two types of liver cancers—hepatocellular carcinoma and cholangiocarcinoma. We also investigate their distributions in relation to liver zonation. These observations provide deep insights into previously unrecognized spatial and temporal regulation of ALDHs in liver zonation.

Introduction

The liver is composed of many small functional units known as liver lobules. Hepatocytes, bile ductular epithelial cells, hepatic stellate cells (HSCs), Kupffer cells, and sinusoidal endothelial cells reside in the liver lobules and participate in homeostasis. Hepatocytes are the major functional cells, and they are stacked one by one in hepatic cords radiating from the central veins to the portal triad. Segregation of hepatocytes into different metabolic zones with functions adapted to oxygen and nutrients occurs according to their concentration nutrient gradients from high to low along the blood flow. Due to endogenous or exogenous exposure during substance exchange and metabolism, toxic intermediates can accumulate in the liver, which filters the blood by removing potentially harmful substances using detoxification mechanisms. Failure to remove these toxins can cause hepatotoxicity. Hepatocyte damage can occur by diverse hepatic insults ranging from viral infections to metabolic syndromes, obesity, drug toxicity, and alcohol abuse. Chronic incidence of these pathological conditions recruits inflammatory cells and activates nonparenchymal cells such as HSCs, leading to liver scarring, cirrhosis, and even liver failure. Liver cancers such as hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) may eventually develop due to the profibrotic microenvironments, resulting in a life-threatening condition. Therefore, characterizing detoxification enzymes in the liver has therapeutic potential to reduce hepatic damage and prevent liver injury and cancer development. This review focuses on acetaldehyde dehydrogenases (ALDHs), a group of key enzymes that catalyze the irreversible oxidation of various aliphatic and aromatic aldehydes to the corresponding carboxylic acids. The distribution patterns of these detoxification genes in normal adult livers, liver zonation, HCC, and CCA were also compared using publicly available databases.

Keywords: ALDH; Lipid peroxidation; Liver cancer; Hepatocellular carcinoma; Cholangiocarcinoma.

Abbreviations: ALDH, acetaldehyde dehydrogenase; AMP, adenosine monophosphate; Arg1, arginase 1; CCA, cholangiocarcinoma; EC, vascular endothelial cells; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; TCGA, The Cancer Genome Atlas.

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ALDHs and their functions

ALDHs consist of 24 families in the eukaryotic *ALDH* gene superfamily. Nineteen of them are found in the human genome and belong to the *ALDH1–9*, *ALDH16*, and *ALDH18* families.[1] There are six isotype genes in the *ALDH1* family (*ALDH1A1*, *ALDH1A2*, *ALDH1A3*, *ALDH1B1*, *ALDH1L1*, and *ALDH1L2*). Among them, *ALDH1A1*, *ALDH1A2*, and *ALDH1A3* encode cytosolic enzymes that oxidize retinal and aliphatic aldehydes. ALDH1A1 protein binds to retinaldehyde in great affinity and has been considered a major retinoid acid-metabolizing enzyme.[2] Cytosolic ALDH1A1 also plays a role in acetaldehyde oxidation and alcohol preference

by mediating the gamma-aminobutyric acid synthesis pathway.[3] In the liver, ALDH1A1 has been shown to be a novel determinant of gluconeogenesis and lipid metabolism independent of adiposity.[4] Deletion of the mouse *Aldh1a1* gene significantly attenuates hepatic triacylglycerol synthesis by increasing adenosine monophosphate (AMP)-activated protein kinase alpha activity and decreasing the expression of lipogenic targets of AMP-activated protein kinase alpha. The *ALDH1* family also contains a mitochondrial ALDH1B1 enzyme involved in metabolizing both retinal and acetaldehyde. It has a high affinity to acetaldehyde only secondary to ALDH2 and catalyzes various aldehyde substrates of acetaldehyde and derivatives of lipid peroxidation.[5] ALDH1L1 and ALDH1L2 are other members of the ALDH1 family that can metabolize 10-formyltetrahydrofolate. They are in the mitochondria and cytosol, respectively.

ALDH2 is the only member of the ALDH2 family. This mitochondrial enzyme is primarily responsible for the oxidization of the majority of hepatic acetaldehyde *in vivo*;[6] however, ALDH1A1 and ALDH1B1 also have a detectable affinity to acetaldehyde.[7] The ALDH3 family consists of three endoplasmic reticulum-located enzymes (ALDH3A2, ALDH3B1, and ALDH3B2) and one cytosolic enzyme (ALDH3A1) that is also partially distributed in the nucleus. ALDH3A1 uses aromatic and aliphatic aldehydes as substrates. ALDH3A2 converts fatty aldehydes to fatty acids, while ALDH3B1 mainly oxidizes octanal. It has been reported that the ALDH3 family has a specific substrate spectrum for all members,[8] although substrates for ALDH3B2 presently remain unknown.

ALDH4A1, ALDH5A1, and ALDH6A1 are found in mitochondria and can metabolize glutamate-gamma-semialdehyde, succinate semialdehyde, and malonate semialdehyde, respectively.[9,10] ALDH7A1, located in the cytosol, is responsible for the oxidation of alpha-aminoadipic semialdehyde.[11] Like ALDH7A1, ALDH8A1 is found in the cytosol but is involved with a cytosolic enzyme for retinal metabolism and the kynurenine pathway for tryptophan catabolism.[12] Additionally, ALDH9A1 is also located in the cytosol and metabolizes gamma-aminobutyraldehyde. ALDH16A1 is a transmembrane protein, but its substrate is still unknown. ALDH18A1 is a mitochondrial enzyme and shares similar substrates with ALDH4A1 for metabolizing glutamic gamma-semialdehyde. Most of the ALDH gene families have the cysteine (PS00070) and glutamic acid (PS00687) active site, but ALDH18A1 encodes a bifunctional protein with a glutamate 5-kinase (PS00902) at the N-terminal site and a gamma-glutamyl phosphate reductase (PS01223) at the C-terminal site.[13] Therefore, there is a distal evolutionary connection between ALDH18A1 and other ALDHs.

Pivotal roles of ALDHs also have been documented based on human genetic disorders. Mutations of ALDH1A2 protein at residue 151 from alanine to serine (A151S) or at residue 157 from isoleucine to threonine (I157T) cause congenital heart disease.[14] ALDH1A3 protein with an arginine mutation at residue 89 to cysteine (R89C) is linked to autosomal recessive anophthalmia and microphthalmia, which are rare developmental eye defects occurring in early fetal development.[15] The ALDH1B1 mutant with alanine to valine at position 86 (A86V) is associated with alcohol-induced hypersensitivity.[16,17] A mutation at residue 793 (D793G) in ALDH1L1 protein is correlated with Hodgkin's lymphoma.[18] The mutation at position 504 from glutamic acid to lysine (E504K) in ALDH2 protein is a risk factor for esophageal cancer,[19,20] diabetic cardiomyopathy,[21–23] cardiac dysfunction,[24] Alzheimer's disease,[25] and colorectal cancer.[26,27] The ALDH3A2 mutation at residue 266 from lysine to asparagine (K266N) causes an inherited neurocutaneous disorder known as Sjögren–Larsson syndrome.[28] ALDH4A1 protein with a mutation at residue 352 from serine to leucine (S352L) is correlated with hyperprolinemia type 2, an autosomal recessive disorder of proline metabolism.[29] ALDH5A1 with a mutation at position 301 from lysine to glutamic acid (K301E) disrupts the normal degradation of gamma-hydroxybutyric acid, resulting in a rare metabolic disorder known as gamma-hydroxybutyric aciduria, which is characterized by a highly heterogeneous neurological phenotype ranging from mild to very severe.[30] Substitutions at position 535 from arginine to cysteine (R535C) or position 466 from glycine to arginine (G466R) in ALDH6A1 are associated with demyelination and transient methylmalonic aciduria.[31] Three mutations in ALDH7A1, which include leucine to proline at position 455 (L455P), glutamic acid to glutamine at position 427 (E427Q), and asparagine to leucine at position 301 (N301I), are associated with pyridoxine-dependent epilepsy and folic acid-responsive seizures.[31] The ALDH16A1 mutation from proline to arginine (P527R) causes gout and mast syndrome.[32] ALDH18A1 with a mutation from arginine to glutamine at position 84 (R84Q) results in urea cycle defects characterized by hyperprolinemia, hypoornithinemia, hypocitrullinemia, hypoargininemia, and hyperammonemia.[33]

Furthermore, ALDH enzymes are involved in many vital physiological processes. By binding to substrates for endobiotic and xenobiotic functions, they not only detoxify potentially hazardous aldehydes, but they also mediate antioxidant activities through direct (glutathione-like) and indirect (generating NAD(P)H) actions. Some of them can transform vitamin A into retinoic acid and perform osmoregulatory functions. Moreover, ALDHs can also protect cells against lipid aldehydes in environments with high levels of oxidative stress. One negative implication of this protective activity is that it allows cancer stem cells or other tumor cells to escape drug toxicity, thus causing cancer resistance.

Cell-type expression patterns of ALDHs in the human adult liver

The liver consists of multiple types of cells. About 80% of liver cells are hepatocytes, which maintain the central liver functions of metabolism, biosynthesis, and detoxification. Bile ductular epithelial cells are the other type of parenchymal cells in the liver, and they form bile ducts to carry out bile acid drainage. Vascular endothelial cells lining the blood vessel walls form sinusoids. HSCs are typically vitamin A-storing cells in the space of Disse between the sinusoid and hepatic plates. The residual macrophage cells in the liver are known as Kupffer cells. They are located near the blood vessel walls in sinusoids as part of immune surveillance. Blood cells, including T cells, B cells, and erythroid cells, are also rich in the liver. To examine the expression patterns of *ALDHs* in human adult livers, we took advantage of the public database Human Protein Altas (https://www.proteinatlas.org) and extracted the single-cell expression data of all *ALDHs* except *ALDH1A7* and *ALDH3B2*. Transcript profiling in this database was based on a combination of two transcriptomics datasets (Human Protein Atlas and Genotype-Tissue Expression) that correspond to a total of 14,590 samples from 54 different human normal tissue types, according to Fagerberg *et al*.[34] As shown in Figure 1, hepatocytes are the main cellular source for 12 *ALDH* genes (*ALDH1A1*, *ALDH1B1*, *ALDH2*, *ALDH1L1*, *ALDH9A1*, *ALDH8A1*, *ALDH5A1*, *ALDH6A1*, *ALDH3A1*, *ALDH3A2*, *ALDH7A1*, and *ALDH4A1*). Although *ALDH18A1* and *ALDH9A1* are highly expressed in hepatocytes, these two genes were also detectable in almost all other cell types (B cells, erythroid cells, T cells, bile ductular epi-

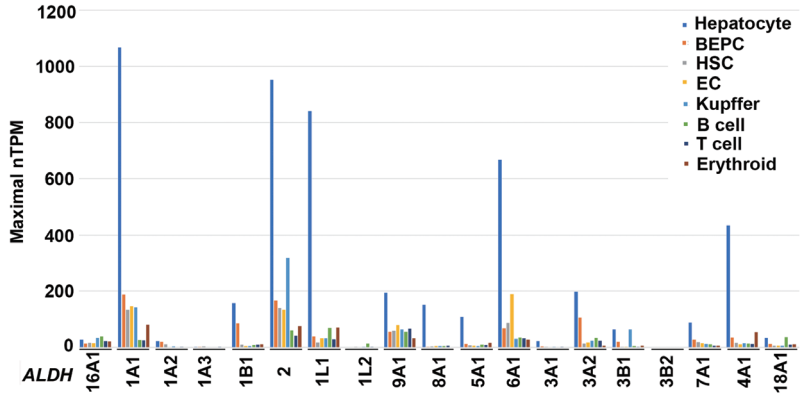

Fig. 1. Expression patterns of *ALDHs* in cells within the human adult liver. Data were extracted based on the maximal transcripts per million (nTPM) for each cell type from the Human Protein Atlas (https://www.proteinatlas.org). ALDH, acetaldehyde dehydrogenase; EC, vascular endothelial cells; HSC, hepatic stellate cell.

thelial cells, endothelial cells, Kupffer cells, and HSCs) in adult human livers. *ALDH16A1* is another gene with wide expression across all cell types in the liver. However, B cells and Kupffer cells have relatively higher levels of *ALDH16A1* than hepatocytes. Notably, some *ALDHs* are not expressed in hepatocytes. For example, *ALDH3B1* is predominantly found in Kupffer cells, while *ALDH1A2* and *ALDH1L2* only have been detected at low levels in B cells, and low levels of *ALDH1A3* have been found in HSCs and bile endothelial progenitor cells. Such differential expression profiles indicate that hepatocytes utilize the majority of ALDHs, whereas other cell types may also use specific enzymes for unique needs during liver homeostasis.

ALDH expression patterns in relation to murine liver zonation

The mammalian liver consists of repeating hexagonally shaped lobules as functional units. As shown in Figure 2a, each liver lobule consists of around 9–12 concentric layers of hepatocytes in mice.[35,36] Liver zonation refers to the phenomenon of spatial and temporal segregation of hepatocytes according to their distinct functions in hepatic cords. Single-molecule fluorescence *in-situ* hybridization can provide sensitivity and dynamic ranges for precise measurement of the mRNA content of hepatocytes in mammalian livers.[37] Combining this technique with single-cell RNA sequencing has revealed the entire transcriptome of thousands of mouse liver cells.[36] In this genome-wide reconstruction of liver zonation, nine layers, starting from the central vein to the portal triads, have been designed to determine the global division of labor in the mammalian liver based on lobule coordinates and zonation landmark genes.[36] Using this strategy, a probabilistic inference algorithm has been developed to compute the likelihood that each cell belongs to any of these layers according to six landmark genes, including the pericentral genes *Glul* and *Cyp2e19* and the periportal genes *Ass110*, *Asl10*, *Alb8*, and *Cyp2f29*.[36] This reconstruction accuracy is strongly dependent on the extent of zonation of tested landmark genes and only weakly dependent on the intralayer cell-to-cell variability. The precision of reconstructed zonation profiles has been validated using single-molecule fluorescence *in-situ* hybridization on 20 genes with diverse profiles and has displayed an excellent overall correspondence between the predicted and measured profiles.[36]

The enzyme arginase 1 (Arg1) is involved in the urea cycle, which is a series of reactions that occur in liver cells near periportal zones. The urea cycle processes excess nitrogen, which is generated when proteins and their building blocks (amino acids) are used by the body. The *Glul* gene product, glutamine synthetase, has opposite patterns that are exclusively located in the first one to two layers of pericentral hepatocytes compared to Arg1. Figure 2b demonstrates the distribution of the periportal enzyme Arg1; the pericentral enzyme glutamine synthetase, which is encoded by Glul; and the perivascular cell marker smooth muscle actin in murine livers. The white dashed lines in Figure 2b indicate hexagon-shaped lobules that are radially polarized to form liver zonation. Considering that key liver genes have been shown to be differentially expressed in different layers of hepatocytes along the liver lobule axis, we examined the distribution of the *Aldh* gene in normal mouse adult livers using extracted data describing the detailed genome-wide reconstruction of the spatial division of hepatocytes in liver zonation.[36] The *Aldh* gene levels were obtained from supplementary table[36] in the zonation matrix for spatial transcriptomics according to Halpern and illustrated by us in heatmaps as shown in Figure 2c–d. These heatmaps for *Aldh* genes were generated based on average values from layer 1 to layer 9 (Fig. 2c–d). We found six different patterns of these genes in the mouse liver zonation, whereas the *Aldh18a1*, *Aldh1a2*, *Aldh1a3*, *Aldh3a1*, and *Aldh3b2* genes were undetectable and were excluded in the analyses. The first pattern showed a peak increase in the pericentral zones. For example, the *Aldh1a1* levels averaged 6.5584E−4 at layer 1 (near the pericentral zone) and 2.3445E−4 at layer 9 (near the periportal zone), showing a roughly 2.797-fold higher level in the pericentral zones than in the periportal zones for the *Aldh1a1* gene. The average *Aldh2* level was 2.1123E−3 at layer 1 and 1.0253E−3 at layer 9, indicating a 2.06-fold higher level of *Aldh2* expression in the pericentral zones versus the periportal zones. *Aldh3a2* expression averaged about 1.1139E−3 at layer 1 and dropped to 4.2813E−05 at layer 9, indicating a 26-fold higher level of *Aldh3a2* expression in the pericentral zones than in the periportal zones. *Aldh16a1* had an average expression of about 8.9078E−05 at layer 1 and 7.1063E−05 at layer 9, a 1.25-fold higher level in *Aldh16a1* in the pericentral zones compared to the periportal zones.

The second pattern of ALDHs showed peak expression at the periportal zones. *Aldh1b1* expression averaged about 3.78886E−06 at layer 1 and 16685E−05 at layer 9, indicating a 24.19-fold increase in the periportal zones compared to the pericentral zones. *Aldh1a7* showed a 1.44-fold increase in the periportal zones compared to the pericentral zones based on its average expression of

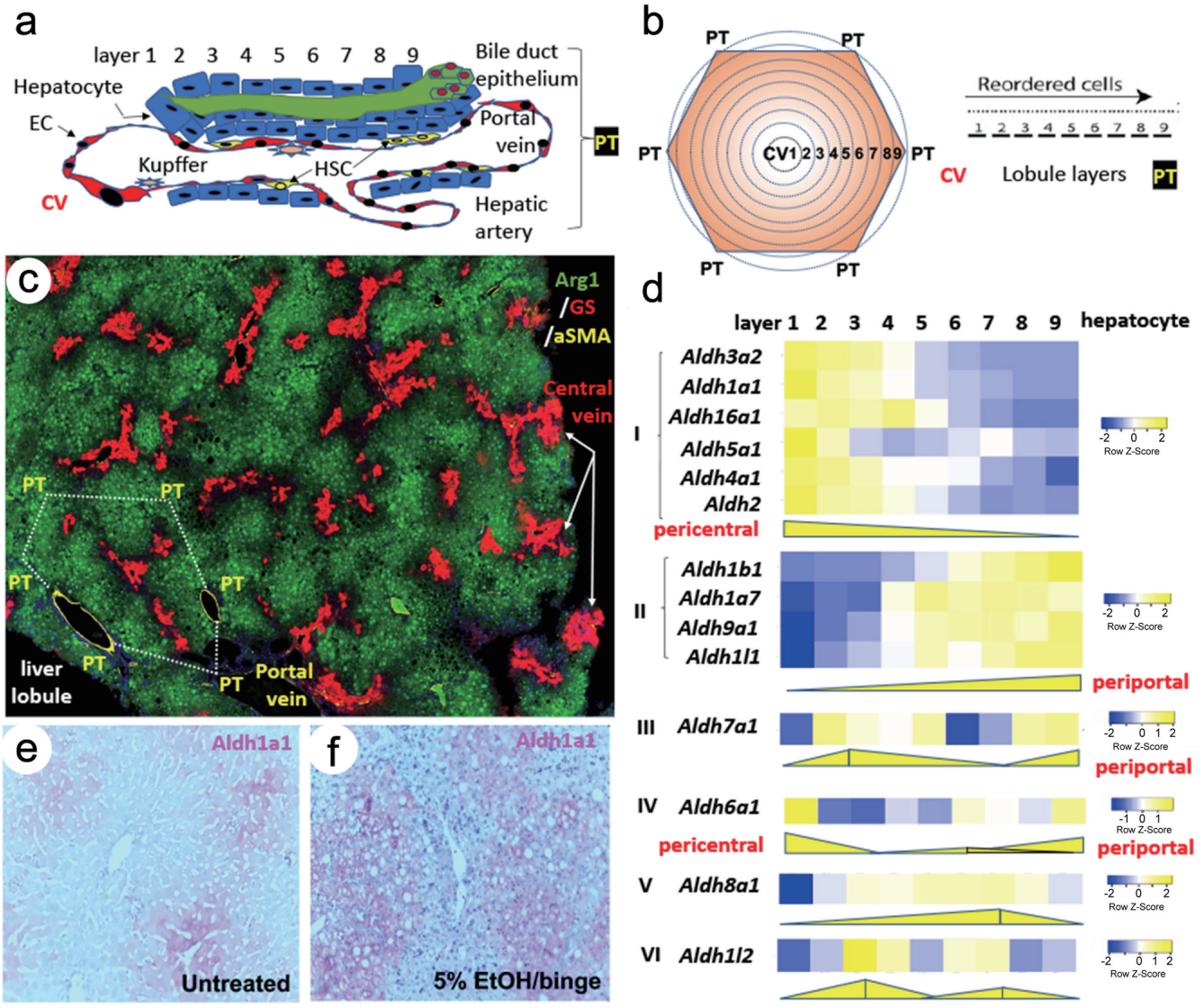

Fig. 2. Expression patterns of *Aldhs* in liver zonation. (a) A cartoon showing the hepatic architecture with layers of hepatic cords, central veins (CV), and portal triads (PT) that contain portal veins, hepatic arteries, and bile ducts. EC: vascular endothelial cells; HSC: hepatic stellate cells. (b) Overview of a liver lobule related to nine layers of hepatocytes in analyses of spatial transcriptomics according to Halpern *et al.*[36] (c) The immunofluorescent staining detects periportal hepatocytes by the Arg1 antibody (green signal), pericentral hepatocytes by the glutamine synthetase (Gs) antibody (red signal), and large hepatic vasculatures by an alpha-smooth muscle actin antibody (yellow signal). The white dashed lines show areas of a liver lobule consisting of 6 PT in the periphery and one CV in the middle. Scar bar: 100 mm. (d) Six types of zonation patterns are found in *Aldhs*. (e) and (f) Immunohistochemistry showed pericentral patterns of Aldh1a1 protein in normal and damaged livers that were exposed to 5% ethanol/binge in chronic and acute liver injury. Magnification: 200×. ALDH, acetaldehyde dehydrogenase.

about 5.91444E−05 at layer 1 and 8.52498E−05 at layer 9. *Aldh9a1* had an average expression of about 1.6432E−4 at layer 1 and 2.3246E−4 at layer 9, demonstrating a 1.415-fold periportal elevation. Lastly, *Aldh1l1* roughly displayed a 1.66-fold periportal increase, with an average expression of about 5.93E−4 at layer 1 and 9.8656E−4 at layer 9.

The third pattern exhibited peak expression in the middle zones with increased levels in the periportal zones. *Aldh1l2* had the highest level in layer 3 (2.149E−07), which was 1150-fold higher than that at layer 1 and 4.28-fold higher than that at layer 9. The fourth pattern showed the lowest expression in the middle zones. *Aldh6a1* had the lowest expression (5.61E−05) in layer 3, which was about a 1.43-fold reduction from layer 1 and a 1.30-fold decrease from layer 9. The fifth pattern had peak expression in the middle zone. *Aldh8a1* expression averaged about 2.0291E−4 at layer 1 and 2.6596E−4 at layer 9, with the peak average expression of about 2.933E−4 found at layer 7. The sixth pattern revealed two expression peaks located in the middle zones. *Aldh1l2* had the highest level in layer 3 (2.149E−07), which was 1150-fold higher than that at layer 1 and 4.28-fold higher than that at layer 9, and another peak expression was at layer 7 (1.18452E−07), which was 633.986-fold higher than that at layer 1 and 2.36-fold higher than that at layer 9.

Spatial sorting enables comprehensive characterization of liver zonation.[38] Transcription dynamics in a physiological process in-

Table 1. Summary of *ALDH* expression in human primary hepatocellular carcinoma

ALDH type	Median value Normal (*n* = 50)	Median value Primary HCC (*n* = 376)	Statistical significance (Normal versus Primary HCC)	Statistical significance of ALDH expression with poor prognosis and survival rates
ALDH1A1	464.669	539.403	Up, $p = 6.103E{-}9$[a]	$p = 0.54$
ALDH1A2	0.502	0.407	Down, $p = 2.623E{-}6$[a]	$p = 0.12$
ALDH1A3	1.436	0.488	$p = 0.595$	$p = 0.26$
ALDH1B1	78.77	39.793	Down, $p = 1.639E{-}5$[a]	$p = 0.039$[a]
ALDH1L1	227.328	113.672	$p = 0.943$	$p = 0.28$
ALDH1L2	0.043	0.081	Up, $p = 7.737E{-}11$[a]	$p = 0.83$
ALDH2	945.96	394.334	Down, $p < 1E{-}12$[a]	$p = 0.081$
ALDH3A1	0.461	1.297	Up, $p = 1.923E{-}6$[a]	$p = 0.98$
ALDH3A2	87.372	87.728	Up, $p = 4.111E{-}6$[a]	$p = 0.93$
ALDH3B1	2.61	3.837	Up, $p = 4.921E{-}13$[a]	$p = 0.038$[a]
ALDH3B2	0.004	0.015	Up, $p = 0.030$[a]	$p = 0.029$[a]
ALDH4A1	198.159	118.372	Down, $p = 7.924E{-}9$[a]	$p = 0.74$
ALDH5A1	34.68	25.422	Down, $p = 0.0254$[a]	$p = 0.049$[a]
ALDH6A1	103.472	29.32	Down, $p < 1E{-}12$[a]	$p = 0.24$
ALDH7A1	64.941	56.968	$p = 0.0636$	$p = 0.041$[a]
ALDH8A1	107.059	34.109	Down, $p = 1.625E{-}12$[a]	$p = 0.029$[a]
ALDH9A1	86.37	67.501	$p = 9.198E{-}4$	$p = 0.94$
ALDH16A1	8.001	14.669	Up, $p < 1E{-}12$ [a]	$p = 0.59$
ALDH18A1	14.497	19.143	Up, $p = 1.624E{-}12$[a]	$p = 0.0014$[a]

[a]*ALDHs* with statistical significance ($p < 0.05$). Data are from The Cancer Genome Atlas database (http://ualcan.path.uab.edu/analysis.html). ALDH, acetaldehyde dehydrogenase; HCC, hepatocellular carcinoma.

dicate that β-catenin signaling directs liver metabolic zonation.[39] *ALDH3A1* expression is not detected in any layers, as indicated in supplementary table by Halpern,[36] but overexpression of this gene has been reported in HCC with the Wnt/β-catenin pathway.[40] Considering that the Wnt/β-catenin pathway controls pericentral genes, this regulation of *ALDH3A1* by Wnt/β-catenin suggests that this enzyme likely is induced by pericentral genes during HCC development. In addition, *ALDH1A1* can be regulated by the Wnt/β-catenin pathway.[41] It is easy to speculate that the pericentral localization of this gene results from the regulation by the Wnt/β-catenin pathway in normal mouse livers. Our recent data have demonstrated potential regulation of the mouse *Aldh1a1* by Yes-associated protein during alcohol-related hepatocyte damage.[42] Moreover, we found Aldh1a1 localization in the pericentral zones in normal mouse livers (Fig. 2e). When mice were exposed to a 5% ethanol-containing Liber Dicarli liquid diet for 10 days followed by a binge (5 mg/g body weight), according to Dr. Bin Gao's National Institute on Alcohol Abuse and Alcoholism model,[43] we observed increased staining of Aldh1a1 in the pericentral zones of the ethanol-damaged livers (Fig. 2f). It is conceivable that both the Yes-associated protein and Wnt/β-catenin pathways are involved in regulating *Aldh1a1* during alcoholic liver disease.

ALDHs in HCC

HCC is the most frequently diagnosed type of liver cancer with a poor prognosis and no effective treatments. Surveillance Epidemiology End Results have reported that HCC is the fastest-growing cause of cancer-related deaths in the United States since the early 2000s.[44,45] To understand the expression patterns of *ALDHs* in HCC, we extracted The Cancer Genome Atlas (TCGA) data and identified altered patterns of the *ALDH* genes. In the upregulated groups, we found a 1.16-fold upregulation of the *ALDH1A1* gene in primary human HCC compared to normal healthy livers (Table 1). This observation is consistent with previous reports about the identification of ALDH1 in metabolic and gene expression profiles that confer cytotoxicity in HepG2 liver cancer cells.[46] ALDH1 activity also has been identified in rabbit hepatic VX2 tumors.[47] In addition, ALDH1A1 protein has been found to stabilize the transcription factor GLI family zinc finger 2 (Gli2) and enhance the Hedgehog signaling in HCC.[48] ALDH1A1 can also crosstalk with insulin growth factor binding protein 1 in liver metastasis from colorectal cancer.[49] Overexpression of the *ALDH1A1* gene has been observed to be in differentiated cells but not in cancer stem/progenitor cells in HCC.[50] High *ALDH1A1* expression is associated with a 57-month recurrence-free survival in hepatitis B virus-related HCC patients.[5] Moreover, *ALDH3A1* overexpression has been identified in HCC with the Wnt/β-catenin pathway.[40] Consistent with this report, we found a 2.8-fold increase in the *ALDH3A1* expression in HCC after analyzing the TCGA database (Table 1). ALDH18A1 is another member of metabolic pathways regulating HCC.[51] The bifunctional *ALDH18A1* gene controls the conversion of glutamate to glutamate 5-semialdehyde in the biosynthesis of proline, ornithine, and arginine. This metabolic axis can support HCC cell survival by modulating hypoxia-inducible factor 1-al-

pha stability in response to hypoxia.[52] *ALDH18A1* also has been identified as a metabolism-related gene in cholesterol-associated nonalcoholic steatohepatitis-HCCs in mice and humans.[53] Furthermore, *ALDH18A1* upregulation in liver cancer of both human and animal models is associated with the reprogramming of mitochondrial proline metabolism with pyrroline-5-carboxylate reductase as a potential mechanism of action for the proline pathway in cancer development.[54] Reducing H3K18Ac and H3K27Ac levels at the promoter regions of amino acid metabolism and nucleotide synthesis enzyme genes including *ALDH18A1* have been found in Huh7 liver cancer cells.[55] In agreement with these reports, we found a 1.32-fold increase in the *ALDH18A1* gene in 372 primary HCCs from the TCGA database (Table 1). Patients with high levels of this gene exhibited decreased survival rates than those exhibiting lower levels (Table 1). These observations support the protumorigenic role of the *ALDH18A1* gene in HCC development. Although *ALDH1L2*, *ALDH3A2*, *ALDH3B1*, *ALDH3B2*, and *ALDH16A1* were upregulated in our analyses of the TCGA database (Table 1), there is no report in the literature about the involvement of these genes in HCC. Nevertheless, we found that *ALDH3B1* and *ALDH3B2* were not only upregulated but also associated with a poorer prognosis in HCC patients with high levels of these two genes (Table 1). These observations indicated undiscovered protumorigenic activities of *ALDH3B1* and *ALDH3B2* in the liver.

On the other hand, we identified downregulated groups in primary human HCC after analyzing the TCGA database. These downregulated genes included *ALDH1A2*, *ALDH1B1*, *ALDH2*, *ALDH4A1*, *ALDH5A1*, *ALDH6A1*, and *ALDH8A1.* Consistent with these observations, *ALDH1A2* has been found to be downregulated in a pathway-guided computational framework to establish a metabolic signature with the capacity for HCC prognosis prediction.[56] We observed 1.233-fold downregulation of *ALDH1A2* in primary human HCC compared to normal healthy livers after analyzing extracted data from the TCGA database (Table 1). ALDH1B1 with high expression has displayed protective roles for HCCs with multiple nodules and high serum alpha-fetoprotein levels.[5] The protective role of Aldh1b1 also has been shown to inhibit ethanol-induced hepatocellular hyperproliferation and tumor development in rodents.[57] Consistent with these previous publications, we found a 1.98-fold downregulation of *ALDH1B1* in primary human HCC (Table 1).

ALDH2 is a potential therapeutic target for liver disease.[58] This enzyme can alleviate alcoholic liver disease by preventing acetaldehyde exposure in the reduction of signal transducer and activator of transcription 1 methylation.[59] It also inhibits oxidative stress and mitochondrial dysfunction in nonalcoholic fatty liver disease.[60] Moreover, ALDH2 activity can be antifibrotic and reduce collagen production by regulating NF-E2-related factor 2/antioxidant responsive element and NF-E2-related factor 2/heme oxygenase-1 signaling pathways.[61,62] However, the *ALDH2* gene is downregulated in many liver diseases. Decreased levels of *ALDH2* have been shown to indicate a poor prognosis in HCC patients.[63] *ALDH2* deficiency also has been linked with a higher risk for the progression of alcohol-associated fibrosis to HCC.[64] Additionally, *ALDH2* loss in hepatocytes has been shown to release copious amounts of oxidized mitochondrial DNAs through extracellular vehicles. Neighboring HCC cells can then take up the extracellular vehicles, containing acetaldehyde, and activate multiple oncogenic pathways that promote carcinogenesis after chronic exposure to alcohol and carbon tetrachloride.[64] Another study suggests a negative correlation between the susceptibility to HCC and ALDH2 expression in an HCC-independent cohort.[65] A dose-dependent link exists between alcohol consumption over time and the risk of HCC individuals with the *ALDH2*1/*2* or *ALDH2*2/*2* genotype.[66] Potential mechanisms by which ALDH2 contributes to HCC advancement arise from the accumulation of acetaldehyde, which causes the increased activation of the AMP-activated protein kinase pathway. Conversely, metastasis is also affected by ALDH2, since modulating the AMP-activated protein kinase pathway affects lipid metabolism and regulates tumor growth and survival.[67] In agreement with these protective roles of *ALDH2* for the liver, we observed a 2.4-fold downregulation of this gene in primary HCC (Table 1). This downregulation supports the concept that loss of protective ALDH2 contributes to HCC development.

ALDH5A1 has been identified as one of eight genes in a prognostic HCC model.[68] We detected a 1.36-fold decrease in this gene in primary human HCC (Table 1). Both the ALDH2 and ALDH5A1 enzymes can oxidize 4-hydroxy-2-nonenal. The loss of ALDH5A1 implies that, like ALDH2, ALDH5A1 has a protective role in the liver, and its loss may contribute to liver damage during HCC development.

ALDH4A1 has been identified as glutamic gamma-semialdehyde dehydrogenase, and ALDH6A1 has altered levels in HCC. We detected 1.674-fold and 3.53-fold decreases of *ALDH4A1* and *ALDH6A1*, respectively, in primary human HCC (Table 1), implicating the loss of the protective roles of these two genes in HCC development. In agreement with this potential function, both genes have been demonstrated as potential molecular signatures for HCC through quantitative analysis of the mitochondrial proteome.[69]

ALDH8A1 is reported as one of eight genes associated with prognosis in a risk score assessment model of HCC patients.[70] We detected a 3.14-fold decrease in this gene in primary human HCC (Table 1). *ALDH1L1* downregulation also has been reported in HCC tumors, and its decreased expression is associated with the poor prognosis of HCC patients.[71] The ALDH1L1 variant rs2276724 and mRNA expression predict postoperative clinical outcomes and are associated with tumor protein p53 expression in hepatitis B virus-related HCC.[72] Knockout of *Aldh1l1* in mice has been demonstrated to reprogram metabolism, thus accelerating HCC.[73] The *ALDH1L1* promoter is extensively methylated in HCC.[74] Additionally, hepatitis B virus-related HCC patients with high *ALDH1L1* gene expression had a better clinical outcome. However, we did not observe any statistical significance in the *ALDH1L1* gene between primary HCC and controls, although there was a 2-fold decrease of the *ALDH1L1* gene in human HCC from the TCGA database (Table 1). In all of the decreased gene groups from the TCGA database, we found statistical significance of a poor prognosis for HCC patients who expressed decreased levels of *ALDH1B1*, *ALDH5A1*, *ALDH7A1*, and *ALDH8A1* (Table 1). These results suggest that the loss of expression of these genes in HCC patients was correlated with worse survival rates. Therefore, these genes can be considered promising diagnostic and prognostic markers as well as potential drug targets.

ALDHs and CCA

CCA is a type of liver cancer arising from the epithelium lining the intrahepatic or extrahepatic biliary ducts.[75] Intrahepatic CCA is classified as peripheral tumors formed in the bile ducts inside the liver, and it accounts for less than 10% of annual CCA cases.[76,77] Hilar or perihilar CCA occurs in the bile ducts just outside of the liver. Distal CCA is also extrahepatic and can arise in the portion of the bile duct nearest the small intestine. Despite different locations, ALDHs have been considered to be molecular markers of CCA stem cells.[78,79] To determine whether there are any alterations of *ALDHs* in CCA, we compared the levels of these genes in CCA tumors after analyzing

Table 2. Summary of *ALDH* expression in human primary cholangiocarcinoma

ALDH type	Median value Normal (*n* = 9)	Median value Primary CC (*n* = 36)	Statistical significance (Normal versus Primary CCA)	Statistical significance for ALDH expression with poor prognosis and survival rates
ALDH1A1	417.329	108.242	Down, *p* = 4.806E−6[a]	*p* = 0.37
ALDH1A2	0.6	1.004	Up, *p* = 0.0487[a]	*p* = 0.36
ALDH1A3	1.275	4.565	Up, *p* = 1.828E−4[a]	*p* = 0.82
ALDH1B1	51.154	30.142	*p* = 0.09586	*p* = 0.2
ALDH1L1	263.865	6.489	Down, *p* = 2.1904E−04[a]	*p* = 0.25
ALDH1L2	0.078	0.565	Up, *p* = 8.0222E−08[a]	*p* = 0.62
ALDH2	1,027.483	122.616	Down, *p* = 6.883E−15[a]	*p* = 0.28
ALDH3A1	0.517	0.603	*p* = 3.528E−01	*p* = 0.15
ALDH3A2	90.025	68.186	*p* = 2.155E−01	*p* = 0.27
ALDH3B1	2.728	23.116	Up, *p* = 3.751E−06[a]	*p* = 0.85
ALDH3B2	0.011	0.508	Up, *p* = 1.816E−03[a]	*p* = 0.012 (high level less survival)[a]
ALDH4A1	186.239	36.732	Down, *p* = 7.043E−10[a]	*p* = 0.35
ALDH5A1	39.281	11.463	Down, *p* = 6.481E−12[a]	*p* = 0.58
ALDH6A1	88.982	7.107	Down, *p* = 1.438E−12[a]	*p* = 0.56
ALDH7A1	63.593	32.341	Down, *p* = 4.447E−04[a]	*p* = 0.84
ALDH8A1	108.593	2.474	Down, *p* < 1E−12[a]	*p* = 0.44
ALDH9A1	84.213	45.997	Down, *p* = 9.460E−03[a]	*p* = 0.47
ALDH16A1	7.919	30.301	Up, *p* = 8.576E−12[a]	*p* = 0.72
ALDH18A1	10.822	33.843	Up, *p* = 2.610E−10[a]	*p* = 0.5

[a]*ALDHs* with statistical significance ($p < 0.05$). The Cancer Genome Atlas dataset "Cholangiocarcinoma" was explored (http://ualcan.path.uab.edu/analysis.html). Data regarding the mRNA levels or relationship between patients' survival rate were obtained to search the genes of interest. ALDH, acetaldehyde dehydrogenase; CCA, cholangiocarcinoma.

the TCGA database from 36 CCA cases. As shown in Table 2, *ALDH1A1*, *ALDH2*, *ALDH1L1*, *ALDH9A1*, *ALDH8A1*, *ALDH5A1*, *ALDH6A1*, *ALDH7A1*, and *ALDH4A1* were downregulated in primary CCA. Similarly, Wang *et al.* have found downregulation of *ALDH1A1*, *ALDH3A2*, *ALDH4A1*, *ALDH6A1*, and *ALDH18A1*; whereas *ALDH3B1* and *ALDH3B2* are highly induced in tumor tissues compared with the peritumor tissues.[80] Downregulation of the *ALDH1A1* and *ALDH6A1* genes is common in CCA from both analyses based on the TCGA data and the study by Wang *et al.*[80] The mechanism of *ALDH1A1* downregulation is known to involve transcriptional regulation by histone H3K27 acetylation in CCA cells.[80] Consistent with these reports, significant downregulation of *ALDH1A1* (3.86-fold decrease) was detected in primary human CCA compared with normal livers in the TCGA database (Table 2). Other discrepancies in the two analyses appear likely to be due to different sample sizes and controls, since Wang *et al.* used eight pairs of CCA samples and adjacent tissues, while TCGA has 36 CCA tumors in comparison to nine normal healthy control livers. Another possibility for the discrepancy may be due to the diversity of extrahepatic and intrahepatic CCAs in the TCGA database and the study by Wang *et al.*[80] Considering that sample sizes are small in the TCGA database, we further searched the publicly available Gene Expression Omnibus dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE26566) that includes 104 freshly frozen CCA tumor samples and 59 matched noncancerous livers obtained from Australia, Europe, and the United States.[81] Significant downregulation of *ALDH1A1*, *ALDH1B1*, *ALDH1L1*, *ALDH2*, *ALDH3A2*, *ALDH4A1*, *ALDH5A1*, *ALDH6A1*, *ALDH7A1*, *ALDH8A1*, and *ALDH9A1* as well as upregulation of *ALDH1A3*, *ALDH3B1*, and *ALDH16A1* were observed (Fig. 3).[81] These observations were consistent with the findings from the TCGA data and the study by Wang *et al.*[80]

ALDH1A3 in CCA plays a vital role in the malignant behavior of CCA and may serve as a new therapeutic target.[82] A positive correlation has been identified between the ALDH1A3 protein expression levels and the cell migration abilities of three CCA cell lines, which has been verified using *ALDH1A3*-overexpressing and *ALDH1A3*-knockdown clones.[83] In addition, lactic acidosis has been shown to upregulate epidermal growth factor receptor and ALDH1A3 expression, leading to the aggressiveness of CCA cells.[84] Given the fact that *ALDH1A3* is protumorigenic, it is not surprising that this gene displayed a 3.58-fold upregulation in CCA (Table 2). We also found upregulation of the *ALDH16A1* (3.83-fold), *ALDH1L2* (7.24-fold), *ALDH3B1* (8.47-fold), *ALDH3B2* (46-fold), and *ALDH18A1* genes (3.13-fold) in CCA tumors (Table 2). *ALDH8A1*, as one of five hub genes, showed higher DNA methylation levels of the promoter in CCA compared with normal liver tissues and has been considered a potential DNA methylation biomarker and therapeutic target in CCA.[85] *ALDH3B2* belongs to the *ALDH3* family of the *ALDH* superfamily.[86] Mammalian *ALDH3* genes (*ALDH3A1*, *ALDH3A2*, *ALDH3B1*, and *ALDH3B2*) encode enzymes of peroxidic and fatty aldehyde metabolism.[87] *ALDH3B2* is found in the endoplasmic reticulum. Although its substrates are unknown, suppression of *ALDH3B2* expression can inhibit the proliferation and clonogenic ability of CCA cells by inducing G1-phase arrest.[88–90] *ALDH3B2* promotes the proliferation and invasion of CCA by increasing the expression of integrin beta1

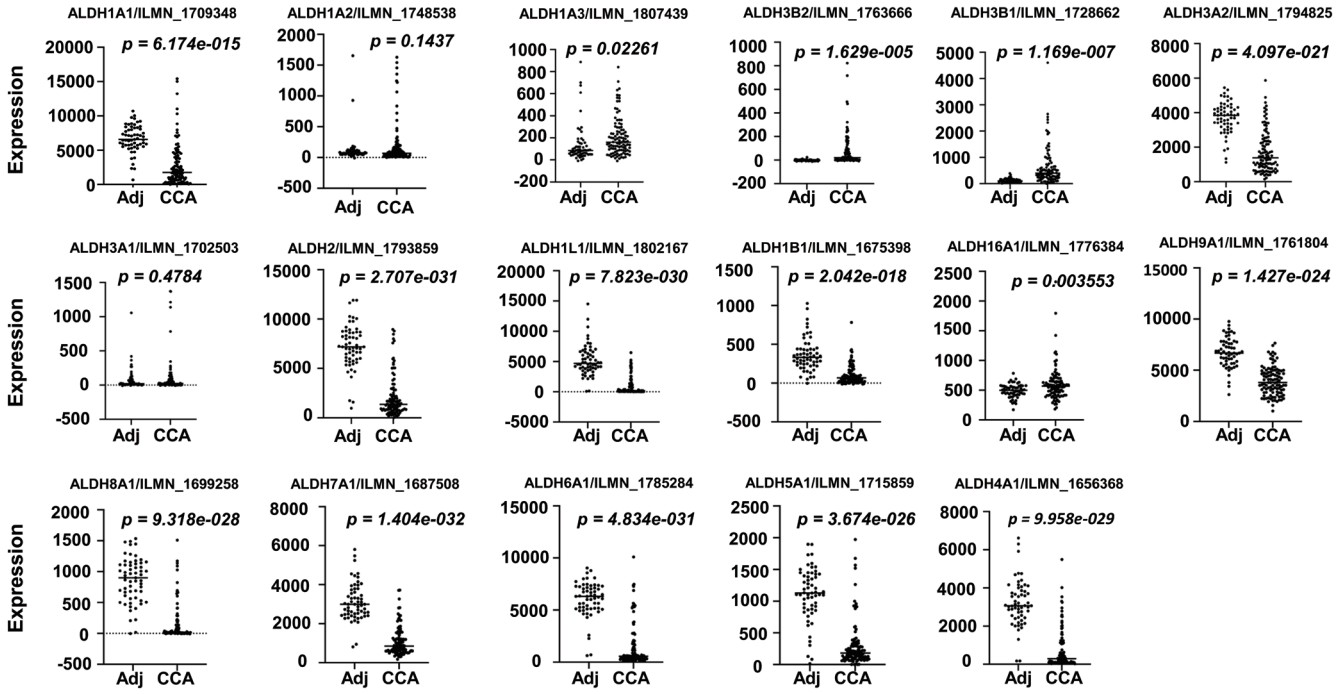

Fig. 3. Altered mRNA levels of *ALDHs* in human cholangiocarcinoma (CCA). Graphed data were extracted from a Gene Expression Omnibus dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE26566) including 104 freshly frozen CCA tumor samples and 59 matched noncancerous adjacent (adj) livers obtained from Australia, Europe, and the United States.[81] The gene ID number in the dataset is labeled following the gene name in each graph. ALDH, acetaldehyde dehydrogenase,

and the phosphorylation levels of downstream c-Jun, ERK 1/2, and p38 MAPK. It has been demonstrated as a prognostic factor of CCA.[91] Despite its undetectable levels in normal human livers (Fig. 1), *ALDH3B2* overexpression is significantly associated with low survival rates in both HCC and CCA patients in our analyses of the TCGA database (Tables 1 and 2). Therefore, *ALDH3B2* is an interesting oncogene worthy of further study.

Conclusions

The 19 detoxification *ALDH* genes exhibit differential spatial and temporal patterns in the liver. In normal conditions, human hepatocytes express *ALDH1A1*, *ALDH1B1*, *ALDH2*, *ALDH1L1*, *ALDH9A1*, *ALDH8A1*, *ALDH5A1*, *ALDH6A1*, *ALDH3A2*, *ALDH7A1*, and *ALDH4A1*. Among them, *ALDH3A2*, *ALDH1A1*, *ALDH16A1*, *ALDH5A1*, *ALDH4A1*, and *ALDH2* are predominately localized in the pericentral zones. In contrast, *ALDH1B1*, *ALDH1A7*, *ALDH9A1*, and *ALDH1L1* are mainly expressed in the periportal zones. *ALDH7A1* has two peaks in layer 2 and the periportal zone, respectively. *ALDH6A1* has peak levels at both the periportal and pericentral areas. *ALDH8A1* and *ALDH1L2* have the lowest expression in both the periportal and pericentral zones, but *ALDH8A1* has a peak in the middle zones, and *ALDH1L2* has two peaks in the middle zones. Upregulation of *ALDH16A1*, *ALDH1A1*, *ALDH1B1*, *ALDH1L2*, *ALDH3A1*, *ALDH3A2*, *ALDH3B1*, *ALDH3B2*, and *ALDH18A1* occur in HCC; whereas *ALDH1A2*, *ALDH2*, *ALDH8A1*, *ALDH5A1*, *ALDH6A1*, and *ALDH4A1* are downregulated in HCC. Loss of *ALDH8A1* and *ALDH5A1* as well as upregulation of *ALDH1B1*, *ALDH3B1*, *ALDH3B2*, and *ALDH18A1* are associated with a poor prognosis and low survival rates in HCC patients. Moreover, the upregulation of *ALDH3B2* is associated with a poor prognosis and low survival rates in CCA patients. These altered expression patterns demonstrate the deregulation of *ALDHs* in the development of HCC and CCA. Whether there are additional changes of the deregulated *ALDHs* during liver injury and cancer development warrants further investigation. Further understanding of *ALDH* genes in the liver, in particular their relation to liver zonation, may help us to develop more accurate and personalized strategies for the treatment of liver diseases such as HCC and CCA.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

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