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Original Article

Identification of alcohol dehydrogenase as a potential prognostic marker in HBV-related hepatocellular carcinoma

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Abstract: Objective: Studies have shown that alcohol dehydrogenase (ADH) expression is associated with cancer risk. This study investigated the prognostic value of *ADH* gene expression in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Materials and methods: Microarray analysis and survival profiles of HBV-related HCC from GSE14520 were used to assess the association between *ADH* gene expression and patient outcome. Statistical correlations between *ADH* gene expression profiles and predefined gene signatures were investigated by gene set enrichment analysis (GSEA). Results: A total of 218 HBV-related HCC patients and six *ADH* genes were examined. *ADH* mRNA expression level was markedly reduced in HBV-related HCC tumor tissue. *ADH1C* and *ADH5* overexpression in tumor tissue was significantly decreased the risk of tumor recurrence [adjusted P = 0.005, adjusted hazard ratio (HR) = 0.581, 95% confidence interval (CI) = 0.398-0.848 and adjusted P = 0.025, adjusted HR = 0.658, 95% CI = 0.455-0.950, respectively], whereas *ADH1A*, *ADH1C*, and *ADH6* overexpression was associated with decreased risk of cancer-related death in HBV-related HCC patients (adjusted P = 0.035, adjusted HR = 0.614, 95% CI = 0.389-0.967; adjusted P = 0.024, adjusted HR = 0.588, 95% CI = 0.371-0.933; and adjusted P = 0.001, adjusted HR = 0.449, 95% CI = 0.282-0.715; respectively). GSEA showed that *ADH1A* and *ADH6* were significantly related to liver cancer survival, whereas *ADH1C* was significantly associated with liver cancer. Conclusions: Upregulation of *ADH* genes (*ADH1A*, *ADH1C*, *ADH5*, and *ADH6*) may have protective effects in HBV-related HCC patients after hepatectomy. Our findings suggest that these genes are potential prognostic markers for HBV-related HCC patients.

Keywords: Prognosis, alcohol dehydrogenase, hepatocellular carcinoma, hepatitis B virus

Introduction

Alcohol abuse, aflatoxin B1, and hepatitis B virus (HBV) and hepatitis C virus infection are the major causes of hepatocarcinogenesis [1, 2]. HBV can cause chronic infection and increase the risk of death from cirrhosis and liver cancer [3]. Approximately 650,000 people die from HBV-related cirrhosis and liver cancer out of a total of 780,000 individuals who die from hepatitis B infection each year [4]. Hepatitis B is most prevalent in sub-Saharan Africa and East Asia including China, where between 5%-10% of the adult population is chronically infected [5-9]. Over half of new liver cancer cases and death from liver cancer in 2012 occurred in China [10], where liver cancer ranks the fourth among the main causes of male cancer-related death [11] and has an age-standardized 5-year relative survival rate of 10.1% [12]. The majority of liver cancer cases are diagnosed as hepatocellular carcinoma (HCC) [13].

The correlation between HBV infection and HCC is well documented, but the link between alcohol metabolism and HCC remains unknown. Patients with HBV-related cirrhosis who drink excessively are at increased risk of HCC as compared to those with HBV infection or alcoholism alone [14]. Alcohol dehydrogenase (ADH) isozymes catalyze the conversion of alcohols to

the corresponding aldehydes, which is a key step in alcohol metabolism [15]. Dysregulation of ADH activity can lead to various diseases, including liver cancer [16-18].

Previous studies have shown that ADH is associated with increased risk of cancers including liver cancer, but few studies have examined the prognostic value of ADH expression in HCC patients [19]. We addressed this in the present study in HBV-related HCC patients after hepatectomy.

Materials and methods

Sources of data

The microarray dataset of HBV-related HCC was obtained from the NCBI gene expression omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database GSE14520, which includes human HCC mRNA expression and corresponding survival profiles. There were 247 HCC patients in the GSE14520 dataset; information on disease-free survival (DFS) and overall survival (OS) as well as the status of events was available for 242 of these patients. A total of 218 HCC patients from the GSE14520 supplementary file with clear evidence of HBV infection and complete follow-up profiles were analyzed in the current study.

Data processing

Data were analyzed with Expression Console software (http://www.affymetrix.com/estore/index.jsp). Probe signal values were converted to log2 values, and annotated genes were analyzed using the corresponding Affymetrix HT Human Genome U133A and Human Genome U133A_2 array annotation files. A multi-array average algorithm was used for normalization of *ADH* mRNA expression data.

Bioinformatics and correlation analysis

We investigated the functions and associations of *ADH* genes using multiple bioinformatics approaches. The relative expression levels of *ADH* genes in multiple normal tissues were determined with GTEx Portal (http://www.gtexportal.org/home/). Co-expression analysis was carried out using GeneMANIA (http://www.genemania.org/). The Database for Annotation, Visualization, and Integrated Discovery (DAVID) v.6.7 (https://david.ncifcrf.gov/tools.jsp) was used to annotate input genes, classify gene functions, identify gene conversions, and carry out Gene Ontology (GO) term analysis. Pearson's correlation coefficient was used to evaluate correlations among genes.

Survival analysis

Samples were divided into two groups according to gene expression levels in tumors. The high expression group consisted of samples in which gene expression levels were above the median value, and the low expression group comprised the remaining samples. DFS and OS were analyzed in the two groups. We also stratified the analysis based on associations between gene expression and clinical features in OS and DFS. Age, gender, cirrhosis, Barcelona Clinic Liver Cancer (BCLC) stage, and serum α-fetoprotein (AFP) level were adjusted in the multivariate Cox proportion haphazard regression analysis.

Gene set enrichment analysis (GSEA)

Tumor tissue samples were divided into high and low gene expression groups. The effect of tumor gene expression level on biological pathways were analyzed by GSEA v2.2.2 (http://software.broadinstitute.org/gsea/index.jsp), with the Molecular Signatures Database (MSigDB) of c2 (curated gene sets: c2.all.v5.1.symbols.gmt) used as a reference gene set. The number of permutations was set at 1000. Genes of the *ADH* family that were significantly associated with DFS or OS were included in the GSEA analysis. Enrichment results satisfying nominal $P<0.05$ with a false discovery rate (FDR) <0.25 were considered statistically significant.

Statistical analysis

Survival analysis was carried out using the Kaplan-Meier method with the log-rank test to compare clinical factors and gene expression groups. Cox proportional hazards regression analysis was used to calculate the crude or adjusted hazard ratio (HR) and 95% confidence interval (CI) in uni- and multivariate analyses. A P value <0.05 was considered statistically significant. Data were analyzed with SPSS v.20.0 software (IBM, Chicago, IL, USA).

Table 1. Clinical characteristics of HBV-related HCC patients

Variables	DFS				OS			
	Events/Total	MST (months)	HR (95% CI)	Log-rank P	Events/Total	MST (months)	HR (95% CI)	Log-rank P
Age (years)				0.736				0.639
≤ 60	100/179	41.6	1		70/179	NA	1	
> 60	21/39	50.0	0.922 (0.576-1.477)		14/39	NA	0.872 (0.491-1.548)	
Gender				0.015				0.15
Female	10/29	NA	1		8/29	NA	1	
Male	111/189	37.9	2.185 (1.143-4.175)		76/189	NA	1.696 (0.818-3.516)	
Cirrhosis				0.026				0.026
No	5/17	NA	1		2/17	NA	1	
Yes	116/201	36.6	2.662 (1.088-6.521)		82/201	NA	4.294 (1.056-17.465)	
Tumor size&				0.072				<0.001
≤ 5 cm	76/141	51.1	1		46/141	NA	1	
> 5 cm	45/76	29.9	1.402 (0.968-2.029)		38/76	53.3	2.083 (1.353-3.207)	
Multinodular				0.254				0.035
Single	92/170	49.1	1		59/170	NA	1	
Multiple	29/48	26.9	1.275 (0.839-1.937)		25/48	47.9	1.645 (1.0030-2.628)	
BCLC stage				<0.001				<0.001
0	6/20	NA	1		2/20	NA	1	
A	77/147	51.1	2.098 (0.914-4.815)		48/147	NA	3.997 (0.971-16.448)	
B	17/24	26.9	4.090 (1.604-10.431)		14/24	46.1	9.609 (2.177-42.423)	
C	21/27	8.9	6.1554 (2.474-15.310)		20/27	13.6	19.139 (4.453-82.261)	
TNM stage				<0.001				<0.001
I	37/92	NA	1		20/92	NA	1	
II	49/77	28.7	1.964 (1.280-3.013)		32/77	NA	2.233 (1.276-3.906)	
III	35/49	18.0	3.116 (1.955-4.968)		32/49	18.0	5.405 (3.070-9.514)	
CLIP stage				0.002				<0.001
0	47/95	53.0	1		26/95	NA	1	
1	41/76	40.1	1.215 (0.799-1.848)		27/76	NA	1.406 (0.820-2.410)	
2/3/4/5	33/47	19.6	2.185 (1.397-3.418)		31/47	26.9	3.662 (2.167-6.190)	
Serum AFPφ				0.449				0.063
≤ 300 ng/ml	65/118	48.0	1		40/118	NA	1	
> 300 ng/ml	55/96	35.2	1.149 (0.802-1.645)		43/96	NA	1.500 (0.975-2.308)	

Notes: &Information of tumor size was unavailable in 1 patients; φInformation of serum AFP was unavailable in 4 patients; BCLC, Barcelona Clinic Liver Cancer; TNM, Tumor Node Metastasis; CLIP, Cancer of the Liver Italian Program; AFP, alpha-fetoprotein; MST, median survival time; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Results

Clinical features and outcomes

A total of 218 HBV-related HCC patients with complete follow-up profiles were recruited for the study; the clinical characteristics are summarized in **Table 1**. At the time of analysis, 84/218 patients (38.5%) had died, and 121/218 (55.5%) showed tumor recurrence. BCLC, tumor-node-metastasis (TNM), and Cancer of the Liver Italian Program (CLIP) stages differed between these two patient groups (all P<0.01; log-rank test). Advanced disease stage was associated with increased risk of HCC recurrence and death in current study population. Tumor size and multiple nodules were significantly associated with OS (P<0.001 and 0.035, respectively; log-rank test) but not with DFS (both P > 0.05; log-rank test). Patients with cirrhosis had higher risk of HCC recurrence and death (both P = 0.026; log-rank test), and male patients had higher risk of recurrence (P = 0.015; log-rank test).

Bioinformatics and correlation analysis

There were six ADH genes in the GSE14520 dataset-i.e., alcohol dehydrogenase 1A (ADH1A), alcohol dehydrogenase 1B (ADH1B), alcohol dehydrogenase 1C (ADH1C), alcohol dehydrogenase 5 (ADH5), alcohol dehydrogenase 6 (ADH6), and alcohol dehydrogenase 7 (ADH7). In normal liver, ADH1A, ADH1C, and ADH6 had

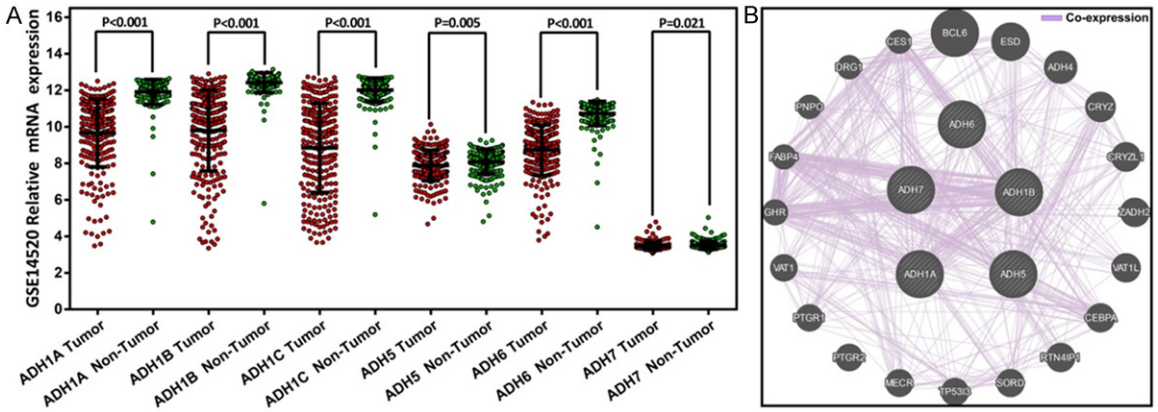

Figure 1. A. Transcript levels of *ADH* genes in HBV-related HCC and adjacent non-tumor tissues. B. Gene interaction networks of *ADH* genes.

Table 2. Correlations between *ADH* gene transcript levels

Genes	ADH1A		ADH1B		ADH1C		ADH5		ADH6		ADH7	
	r	p	r	p	r	p	r	p	r	p	r	p
ADH1A	–	–	0.721	<0.001	0.471	<0.001	0.475	<0.001	0.684	<0.001	0.013	0.853
ADH1B	0.721	<0.001	–	–	0.687	<0.001	0.458	<0.001	0.694	<0.001	0.022	0.75
ADH1C	0.471	<0.001	0.687	<0.001	–	–	0.209	0.002	0.458	<0.001	0.033	0.633
ADH5	0.475	<0.001	0.458	<0.001	0.209	0.002	–	–	0.52	<0.001	-0.073	0.281
ADH6	0.684	<0.001	0.694	<0.001	0.458	<0.001	0.52	<0.001	–	–	0.042	0.541
ADH7	0.013	0.853	0.022	0.75	0.033	0.633	-0.073	0.281	0.042	0.541	–	–

Notes: ADH, alcohol dehydrogenase.

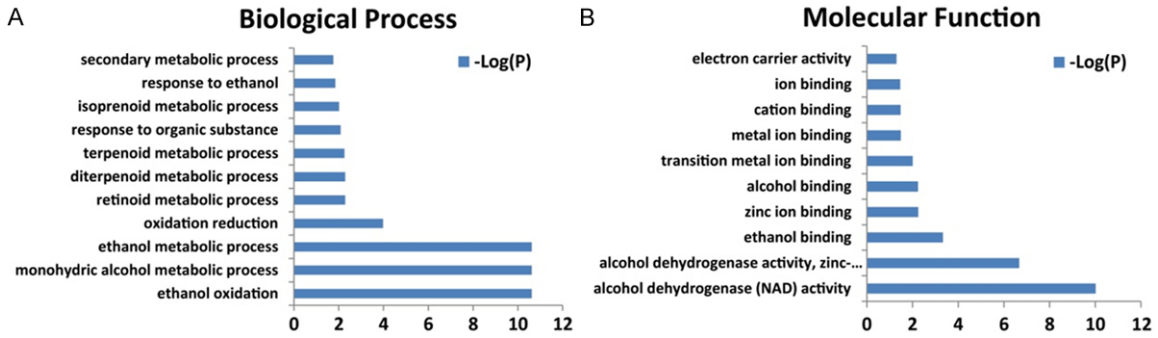

Figure 2. Analysis of enriched GO terms for *ADH* genes carried out using DAVID. A. GO terms of molecular functions. B. GO terms of biological process.

the highest expression, while the remaining ADH genes were also present at high levels (Supplementary Figure 1A-F). A comparison between tumor and adjacent non-tumor tissue revealed that all of ADH gene transcripts were downregulated in HBV-related HCC relative to normal tissue (all P<0.05) (**Figure 1A**). A gene co-expression interaction analysis showed that all ADH genes were co-expressed, constituting a large and complex co-expression network except for ADH1C (**Figure 1B**), which was not recognized by GeneMANIA. A correlation analysis showed that the expression levels of ADH1A, ADH1B, ADH1C, ADH5, and ADH6 were weakly or moderately correlated (all P<0.01; **Table 2**). However, there was no correlation between ADH7 and the other five genes (**Table 2**). A GO term analysis using DAVID revealed that the most highly enriched GO terms associated with ADH genes pertained to ethanol-related biologi-

Table 3. Associations between *ADH* genes and DFS in HBV-related HCC patients

Gene expression	Patients (n = 218)	No. of Event (%)	MST (months)	Crude HR (95% CI)	Crude P	Adjusted HR (95% CI)	Adjusted P§
ADH1A							
Low	109	66	32.6	1		1	
High	109	55	51.1	0.721 (0.504-1.031)	0.073	0.855 (0.588-1.241)	0.409
ADH1B							
Low	109	66	28.2	1		1	
High	109	55	54.8	0.652 (0.456-0.934)	0.020	0.750 (0.508-1.108)	0.148
ADH1C							
Low	109	68	28.4	1		1	
High	109	53	57.7	0.605 (0.422-0.867)	0.006	0.581 (0.398-0.848)	0.005
ADH5							
Low	109	69	28.2	1		1	
High	109	52	57.7	0.581 (0.405-0.833)	0.003	0.658 (0.455-0.950)	0.025
ADH6							
Low	109	66	32.6	1		1	
High	109	55	54.8	0.740 (0.517-1.058)	0.099	0.764 (0.526-1.111)	0.159
ADH7							
Low	109	66	40.1	1		1	
High	109	55	51.1	0.816 (0.570-1.167)	0.265	0.743 (0.515-1.072)	0.113

Notes: §Adjustment for age, gender, cirrhosis, BCLC stage, serum AFP level; ADH, alcohol dehydrogenase; MST, median survival time; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

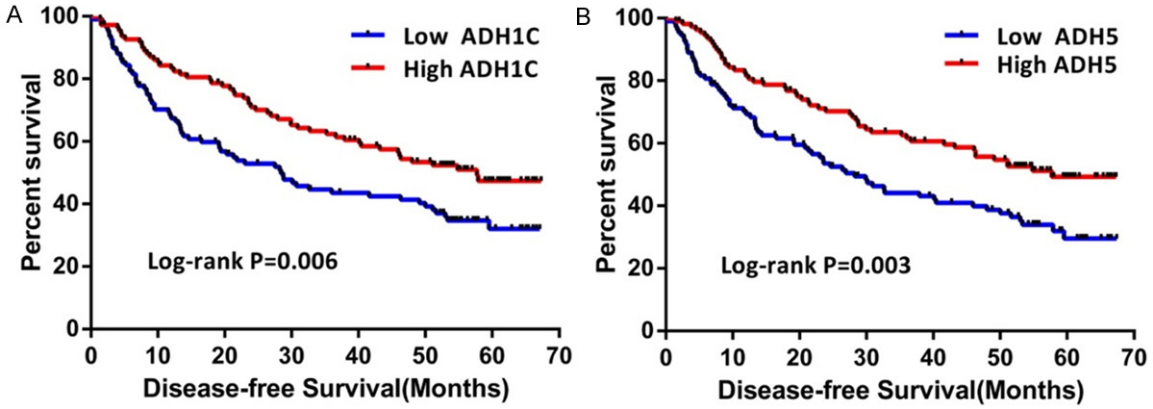

Figure 3. Kaplan-Meier survival curves of HBV-related HCC patients. (A, B) DFS stratified by *ADH1C* (A) and *ADH5* (B) expression.

cal processes, including response to ethanol and ethanol oxidation and metabolism (**Figure 2A**). The top-ranking enriched GO terms for molecular function were significantly associated with alcohol dehydrogenase activity and ethanol and alcohol binding (**Figure 2B**).

Survival analysis

DFS analysis for *ADH* genes showed that overexpression of *ADH1C* and *ADH5* in tumor tissue was associated with reduced risk of tumor recurrence in HBV-related HCC (adjusted P = 0.005, adjusted HR = 0.581, 95% CI = 0.398-0.848 and adjusted P = 0.025, adjusted HR = 0.658, 95% CI = 0.455-0.950, respectively) (**Table 3** and **Figure 3**), as determined with the multivariate Cox proportional hazards regression model after adjusting for age, gender, cirrhosis, BCLC stage, and serum AFP level. OS analysis of ADH genes revealed that high *ADH1A*, *ADH1C*, and *ADH6* transcript levels in tumor tissue reduced the risk of death from HBV-related HCC (adjusted P = 0.035, adjusted

Table 4. Associations between ADH genes and OS in HBV-related HCC patients

Gene expression	Patients (n = 218)	NO. of Event (%)	MST (months)	Crude HR (95% CI)	Crude P	Adjusted HR (95% CI)	Adjusted P§
ADH1A							
Low	109	52	57.9	1		1	
High	109	32	NA	0.507 (0.326-0.789)	0.003	0.614 (0.389-0.967)	0.035
ADH1B							
Low	109	51	54.8	1		1	
High	109	33	NA	0.505 (0.325-0.783)	0.002	0.635 (0.394-1.024)	0.062
ADH1C							
Low	109	50	59.2	1		1	
High	109	34	NA	0.562 (0.363-0.870)	0.010	0.588 (0.371-0.933)	0.024
ADH5							
Low	109	50	57.9	1		1	
High	109	34	NA	0.570 (0.368-0.882)	0.012	0.665 (0.4426-1.037)	0.072
ADH6							
Low	109	55	53..0	1		1	
High	109	29	NA	0.445 (0.283-0.698)	0.0004	0.449 (0.282-0.715)	0.001
ADH7							
Low	109	44	NA	1		1	
High	109	40	NA	0.905 (0.589-1.388)	0.646	0.827 (0.531-1.289)	0.402

Notes: §Adjustment for age, gender, cirrhosis, serum AFP level, BCLC stage; ADH, alcohol dehydrogenase; MST, median survival time; OS, overall survival; HR, hazard ratio; CI, confidence interval.

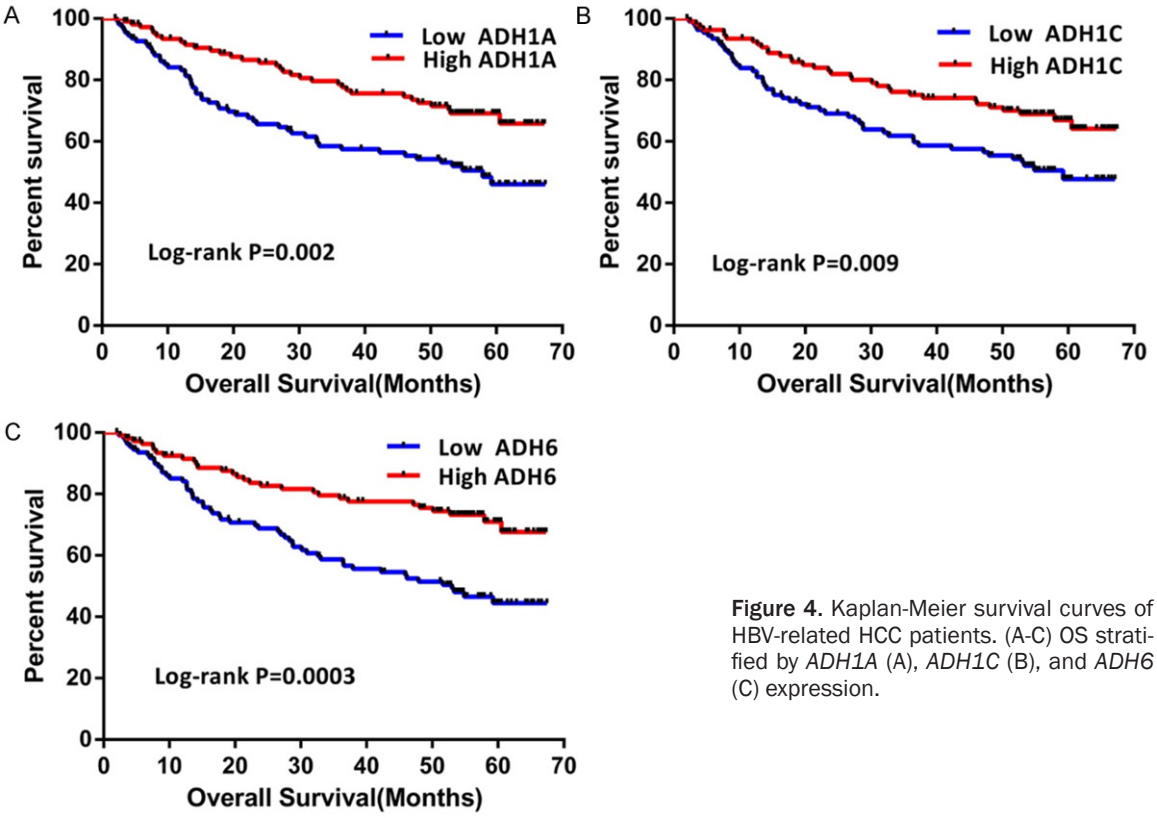

Figure 4. Kaplan-Meier survival curves of HBV-related HCC patients. (A-C) OS stratified by *ADH1A* (A), *ADH1C* (B), and *ADH6* (C) expression.

HR = 0.614, 95% CI = 0.389-0.967; adjusted P = 0.024, adjusted HR = 0.588, 95% CI = 0.371-0.933; and adjusted P = 0.001, adjusted HR = 0.449, 95% CI = 0.282-0.715, respectively) (**Table 4** and **Figure 4**), as shown by the multivariate Cox proportional hazards regression

Table 5. Stratified analysis of associations between *ADH1C* or *ADH5* and DFS in HBV-related HCC patients

Variables	Patients (n = 218)	ADH1C		Adjusted HR (95% CI)	Adjusted P§	ADH5		Adjusted HR (95% CI)	Adjusted P§
		Low	High			Low	High		
Age (years)									
≤ 60	179	91	88	0.673 (0.446-1.017)	0.060	92	87	0.572 (0.381-0.858)	0.007
> 60	39	18	21	0.187 (0.056-0.627)	0.007	17	22	1.165 (0.459-2.952)	0.748
Gender									
Female	29	17	12	0.201 (0.031-1.307)	0.093	10	19	0.621 (0.116-3.328)	0.578
Male	189	92	97	0.599 (0.403-0.888)	0.011	99	90	0.684 (0.466-1.005)	0.053
Cirrhosis									
No	17	8	9	1.797 (0.295-10.956)	0.525	8	9	0.620 (0.097-3.965)	0.614
Yes	201	101	100	0.555 (0.376-0.819)	0.003	101	100	0.674 (0.463-0.983)	0.040
Tumor size&									
≤ 5 cm	141	67	74	0.641 (0.399-1.029)	0.066	63	78	0.492 (0.311-0.777)	0.002
> 5 cm	76	42	34	0.453 (0.228-0.897)	0.023	45	31	0.843 (0.449-1.580)	0.593
Multinodular									
Single	170	83	87	0.511 (0.330-0.790)	0.003	83	87	0.703 (0.459-1.075)	0.104
Multiple	48	26	22	0.813 (0.376-1.754)	0.597	26	22	0.621 (0.284-1.358)	0.233
BCLC stage									
0	20	12	8	0.369 (0.042-3.240)	0.368	8	12	0.094 (0.011-0.831)	0.034
A	147	67	80	0.496 (0.307-0.802)	0.004	70	77	0.688 (0.438-1.082)	0.105
B	24	13	11	1.340 (0.448-4.012)	0.601	14	10	0.408 (0.113-1.467)	0.170
C	27	17	10	0.600 (0.221-1.627)	0.315	17	10	1.026 (0.404-2.604)	0.957
TNM stage									
I	92	38	54	0.539 (0.260-1.117)	0.096	39	53	0.770 (0.391-1.515)	0.449
II	77	42	35	0.474 (0.256-0.879)	0.018	41	36	0.469 (0.252-0.870)	0.016
III	49	29	20	0.883 (0.407-1.917)	0.753	29	20	0.869 (0.417-1.808)	0.707
CLIP stage									
0	95	33	62	0.513 (0.276-0.954)	0.035	41	54	0.690 (0.386-1.234)	0.211
1	76	46	30	0.731 (0.353-1.513)	0.398	40	36	0.565 (0.284-1.122)	0.103
2/3/4/5	47	30	17	0.447 (0.188-1.062)	0.068	28	19	0.731 (0.348-1.535)	0.408
Serum AFPφ									
≤ 300 ng/ml	118	43	75	0.657 (0.390-1.106)	0.114	55	63	0.612 (0.374-1.000)	0.050
> 300 ng/ml	96	66	30	0.473 (0.254-0.880)	0.018	52	44	0.715 (0.411-1.246)	0.236

Notes: &Information of tumor size was unavailable in 1 patients; φInformation of serum AFP was unavailable in 4 patients; §Adjustment for age, gender, cirrhosis, BCLC stage, serum AFP level; BCLC, Barcelona Clinic Liver Cancer; TNM, Tumor Node Metastasis; CLIP, Cancer of the Liver Italian Program; AFP, alpha-fetoprotein; MST, median survival time; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

model with the same adjustments as for the DFS analysis.

Stratification analysis

ADH genes that were significantly associated with DFS and OS were subjected to stratification analysis. In the stratified DFS analysis, high *ADH1C* expression decreased the risk of tumor recurrence among HBV-related HCC patients who were older and male; had cirrhosis and a single tumor with a size > 5 cm; and were BCLC stage A, TNM stage II, CLIP stage 0, and had serum AFP level > 300 ng/ml (**Table 5**). Patients with high *ADH5* expression were younger, had cirrhosis and a tumor ≤ 5 cm in size, were BCLC stage 0 and TNM stage II, and had lower risk of tumor recurrence in HBV-related HCC.

In the stratified OS analysis, upregulation of *ADH1A*, *ADH1C*, and *ADH6* was associated with decreased risk of cancer death among patients with older age, cirrhosis and a single tumor and who were BCLC stage A (**Table 6**). In male HBV-related HCC patients, high *ADH1A* and *ADH1C* levels decreased the risk of cancer death. This protective effect was also observed among

Table 6. Stratified analysis of associations between *ADH1A*, *ADH1C*, or *ADH6* and OS in HBV-related HCC patients

Variables	Patients (n = 218)	ADH1A		Adjusted HR (95% CI)	Adjusted P§	ADH1C		Adjusted HR (95% CI)	Adjusted P§	ADH6		Adjusted HR (95% CI)	Adjusted P§
		Low	High			Low	High			Low	High		
Age (years)													
≤ 60	179	89	90	0.858 (0.524-1.406)	0.543	91	88	0.688 (0.418-1.133)	0.142	94	85	0.526 (0.319-0.869)	0.012
> 60	39	20	19	0.098 (0.017-0.557)	0.009	18	21	0.128 (0.025-0.660)	0.014	15	24	0.198 (0.052-0.749)	0.017
Gender													
Female	29	13	16	0.684 (0.101-4.632)	0.697	17	12	0.131 (0.012-1.445)	0.097	16	13	$1*10^{-6}$ ($1*10^{-7}$-$4.004*10^{161}$)	0.898
Male	189	96	93	0.594 (0.368-0.960)	0.033	92	97	0.635 (0.392-1.028)	0.064	93	96	0.504 (0.312-0.814)	0.005
Cirrhosis													
No	17	7	10	$1.4*10^{-5}$ (10^{-6}-$5.787*10^{248}$)	0.970	8	9	$2.276*10^{5}$ (10^{-6}-$1.510*10^{248}$)	0.970	9	8	$3*10^{-6}$ ($1*10^{-6}$-$2.686*10^{225}$)	0.975
Yes	201	102	99	0.609 (0.384-0.966)	0.035	101	100	0.548 (0.343-0.878)	0.012	100	101	0.462 (0.289-0.737)	0.001
Tumor size&													
≤ 5 cm	141	65	76	0.608 (0.333-1.112)	0.106	67	74	0.664 (0.354-1.247)	0.203	60	81	0.460 (0.243-0.872)	0.017
> 5 cm	76	44	32	0.596 (0.284-1.252)	0.172	42	34	0.472 (0.218-1.025)	0.058	49	27	0.344 (0.157-0.753)	0.008
Multinodular													
Single	170	76	94	0.498 (0.296-0.838)	0.009	83	87	0.555 (0.319-0.964)	0.037	78	92	0.412 (0.238-0.711)	0.001
Multiple	48	33	15	0.762 (0.285-2.041)	0.589	26	22	0.757 (0.313-1.834)	0.538	31	17	0.511 (0.190-1.372)	0.183
BCLC stage													
0	20	9	11	0.303 (0.014-6.793)	0.452	12	8	$7*10^{-6}$ ($1*10^{-6}$-$1.317*10^{273}$)	0.971	9	11	$1*10^{-5}$ ($1*10^{-6}$-$3.005*10^{239}$)	0.968
A	147	65	82	0.539 (0.304-0.958)	0.035	67	80	0.454 (0.245-0.840)	0.012	68	79	0.395 (0.214-0.727)	0.003
B	24	17	7	0.214 (0.037-1.249)	0.087	13	11	1.452 (0.427-4.941)	0.551	16	8	0.389 (0.093-1.631)	0.197
C	27	18	9	0.888 (0.332-2.378)	0.814	17	10	0.776 (0.284-2.123)	0.622	16	11	0.674 (0.260-1.742)	0.415
TNM stage													
I	92	34	58	0.589 (0.234-1.485)	0.262	38	54	0.459 (0.164-1.286)	0.138	37	55	0.467 (0.182-1.200)	0.114
II	77	41	36	0.635 (0.308-1.309)	0.219	42	35	0.418 (0.196-0.892)	0.024	43	34	0.281 (0.115-0.688)	0.006
III	49	34	15	0.698 (0.287-1.698)	0.428	29	20	1.205 (0.540-2.686)	0.649	29	20	0.710 (0.325-1.550)	0.390
CLIP stage													
0	95	38	57	0.534 (0.244-1.170)	0.117	33	62	0.445 (0.197-1.005)	0.051	36	59	0.381 (0.172-0.845)	0.017
1	76	41	35	0.595 (0.255-1.389)	0.230	46	30	0.677 (0.272-1.682)	0.400	45	31	0.490 (0.185-1.301)	0.152
2/3/4/5	47	30	17	0.786 (0.330-1.872)	0.586	30	17	0.543 (0.221-1.335)	0.184	28	19	0.476 (0.209-1.082)	0.076
Serum AFPφ													
≤ 300 ng/ml	118	53	65	0.626 (0.331-1.181)	0.148	43	75	0.702 (0.360-1.367)	0.298	49	69	0.544 (0.279-1.061)	0.074
> 300 ng/ml	96	54	42	0.627 (0.322-1.221)	0.170	66	30	0.479 (0.234-0.979)	0.044	57	39	0.347 (0.171-0.704)	0.003

Notes: &Information of tumor size was unavailable in 1 patients; φInformation of serum AFP was unavailable in 4 patients; §Adjustment for age, gender, cirrhosis, BCLC stage, serum AFP level. BCLC, Barcelona Clinic Liver Cancer; TNM, Tumor Node Metastasis; CLIP, Cancer of the Liver Italian Program; AFP, alpha-fetoprotein; MST, median survival time; OS, overall survival; HR, hazard ratio; CI, confidence interval.

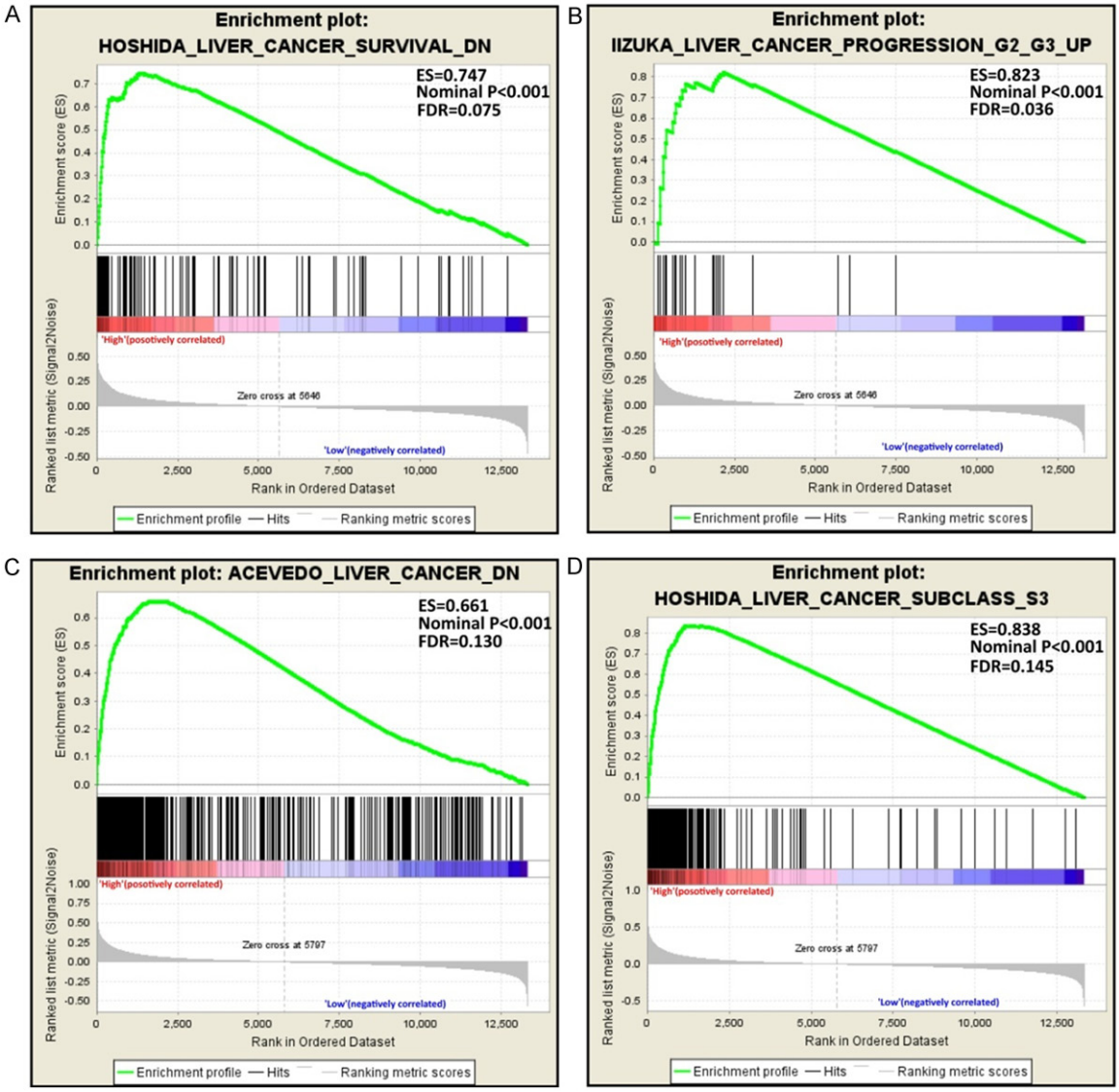

Figure 5. GSEA of *ADH* genes expressed in HBV-related HCC patients. (A-D) Results are shown for high *ADH1A* (A, B) and *ADH1C* (C, D) expression groups in GSE14520.

patients with CLIP stage 0 and serum AFP > 300 ng/ml in high *ADH1C* and *ADH6* expression groups, respectively. High *ADH6* expression was associated with decreased risk of cancer death in patients with any tumor size.

GSEA

To determine whether the expression of *ADH* genes is related to the prognosis of HBV-related HCC patients, GSE14520 expression data were subjected to GSEA, grouping the *ADH* genes that were associated with DFS and OS.Top 20 GSEA enrichment results of each genes are summarized in supplementary material (Supplementary Tables 1, 2, 3, 4). High *ADH1A* expression was significantly associated with liver cancer survival and progression (**Figure 5A** and **5B**), whereas *ADH1C* expression enrichment was significantly related to liver cancer but not progression (**Figure 5C** and **5D**). High *ADH5* and *ADH6* levels in HBV-related HCC were related to liver cancer survival and recurrence (**Figure 6**), although the GSEA results of *ADH5* had an FDR > 0.25, indicating that *ADH5* is not related to these parameters through the GSEA approach.

Discussion

ADH genes are located on 4q23 of chromosome 4; the protein can be divided into five

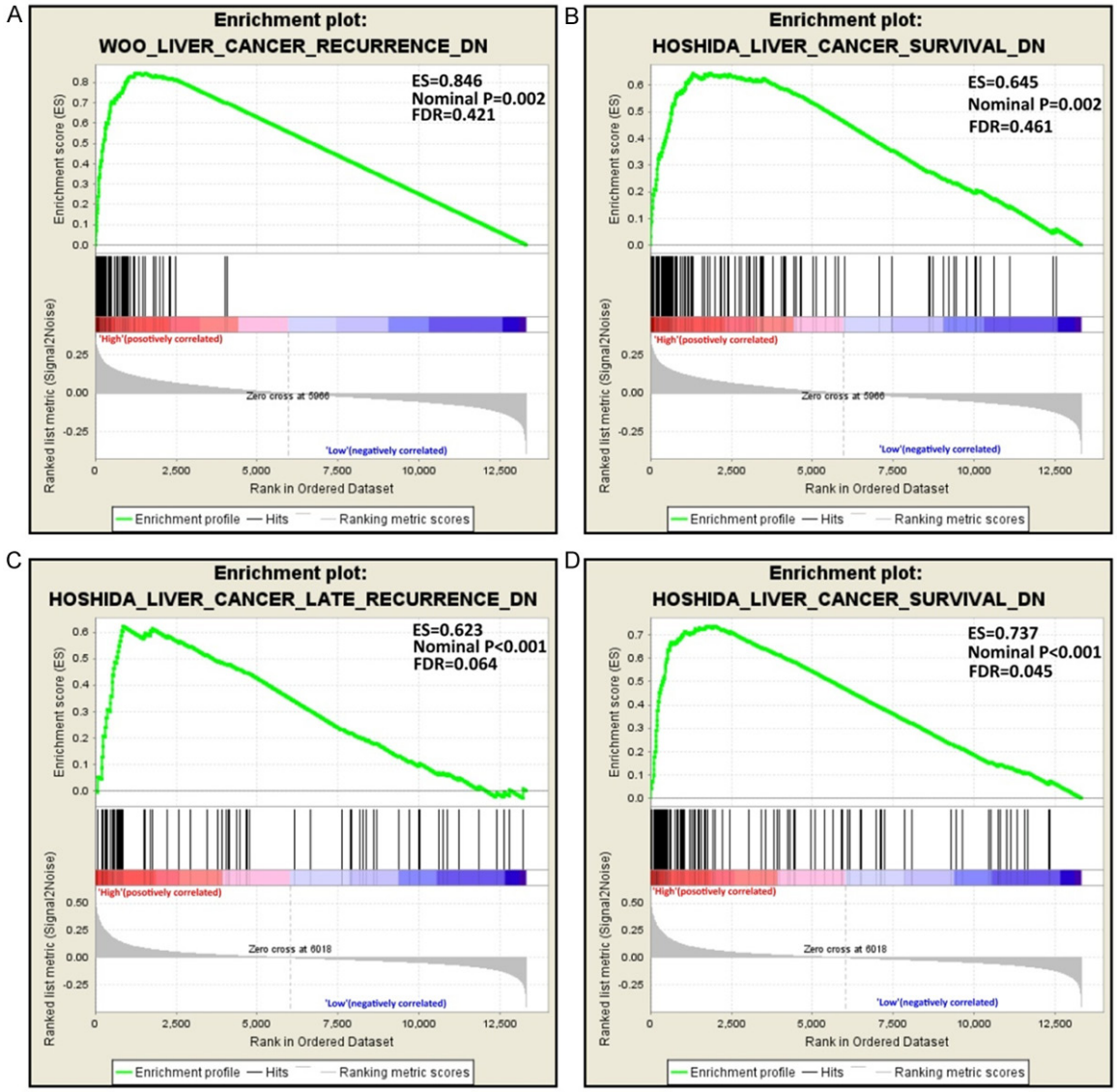

Figure 6. GSEA of *ADH* genes expressed in HBV-related HCC patients. (A-D) Results are shown for high *ADH5* (A, B) and high *ADH6* (C, D) expression groups in GSE14520.

classes based on sequence and structural similarities. Class I ADH isoenzymes consists of α, β, and γ subunits, encoded by *ADH1A*, *ADH1B*, and *ADH1C*, respectively. The *ADH5* gene encodes the χ subunit of a Class III isoenzyme, whereas *ADH6* and *ADH7* encode Class V and IV isoenzymes, respectively, with the latter consisting of the σ subunit [20, 21]. This enzyme family is involved in the metabolism of a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products [22].

Our bioinformatics analysis revealed that class I *ADH* genes were more highly expressed in normal liver and colorectal as compared to other tissues. Previous studies have shown that total ADH and class I ADH isoenzyme activities are elevated in the tissues and serum of patients with liver cancer [17, 18, 23], colorectal cancer (CRC) [24-26], brain cancer [27, 28], renal cell carcinoma (RCC) [29, 30], endometrial cancer [31, 32], cervical cancer [33, 34], breast cancer [35], ovarian cancer [36], and bladder cancer [37], while ADH7 is overexpressed in stomach and esophageal cancers [38]. Therefore, both of the total ADH and class IV ADH isoenzyme were significantly activity in the sera of esophageal cancer [39] and gastric cancer (GC) [40] patients, identical result also been found

in esophageal tumor tissue [41]. Total ADH and ADH III also showed higher activity in the serum and pancreatic cancer tissue of healthy subjects [42, 43]. Thus, expression of ADH isoenzymes can serve as a diagnostic marker for specific types of cancer. Indeed, the utility of ADH I for diagnosis of CRC [26], RCC [30], endometrial cancer [32], and cervical cancer [33] has been reported. Similarly, class III ADH is a potential marker for pancreatic cancer [43], whereas class IV ADH is a marker for gastric [40] and esophageal [44] cancers. Serum ADH activity is also used to assess the function of grafts after liver transplantation [45]. In contrast to ADH isoenzymes, the activity of aldehyde dehydrogenase (ALDH) isoenzyme does not differ between cancer patients and normal subjects; discrepancies between the activities of ADH and ALDH lead to increased acetaldehyde accumulation and decreased acetaldehyde elimination, which is known to promote carcinogenesis [16, 46].

ADH gene polymorphism has been linked to cancer risk [47]. For instance, the rs1230025 polymorphism of *ADH1A* is associated with increased GC cancer risk, which may be exacerbated by alcohol intake [48]. *ADH1B* polymorphisms were related to increased cancer risk in CRC [49], upper aerodigestive tract (UAT) cancer [50], esophageal squamous cell carcinoma (ESCC) [51], and head and neck squamous cell carcinoma (HNSCC) [52] but did not increase the risk of HCC [53, 54] or pancreatic cancer [55].

ADH1C-rs398 Ile350Val is a non-synonymous single nucleotide polymorphism and complete linkage disequilibrium with polymorphisms of Arg272Gln, the alter of them can be used to distinguish the *ADH1C*1* and *ADH1C*2* alleles, which differ in terms of oxidative function [20, 56]. *ADH1C*2*2* has been implicated in the etiology of oral squamous cell carcinoma [57, 58], bladder cancer [59], UAT cancer [60, 61], and CRC [62, 63]. The G allele of *ADH1C*-rs698 also contributes to the risk of HNSCC [52] and UAT cancer [64, 65], although it is not associated with esophageal cancer risk [66]. The rs283411, rs1614972, and rs1789903 polymorphisms of *ADH1C* increased the risk of GC [48] and ESCC [51]. ADH7-rs1573496 was linked to UAT cancer [50] and HNSCC [67], whereas rs1573496 (C) allele carriers who engaged in heavy drinking had higher risk of CRC [68]. A genome-wide association study demonstrated that ADH7-rs17028973 was associated with the risk of developing ESCC [51]. In contrast, *ADH5* and *ADH6* genetic polymorphisms in cancer risk are rarely involved.

The prognostic value of *ADH* genes has rarely been investigated. ADH4 was downregulated in HCC tissue compared to adjacent mucosa both at the mRNA and protein levels, and was linked to lower OS rates [19]. In the present study, we found that *ADH* gene transcripts were downregulated in HBV-related HCC tumor tissue, consistent with previous reports. We speculate that *ADH* genes may have a tumor-suppressor role in HBV-related HCC. Our survival analysis also indicated that decreased *ADH1C* and *ADH5* expression in HBV-related HCC tumor tissue predicted earlier recurrence, whereas *ADH1A*, *ADH1C*, and *ADH6* predicted poor survival. The GSEA in cancer patients revealed that high *ADH1A* and *ADH6* expression was associated with liver cancer survival, and that *ADH1C* was significantly related to liver cancer.

This study had certain limitations. Firstly, the clinical information in the public databases was not comprehensive; as such, confounding factors affecting HBV-related HCC patient prognosis were not included in the Cox proportional hazards regression model. Secondly, due to lacking of information regarding alcohol intake, we were unable to analyze the interaction between this and *ADH* gene expression in terms of patient outcomes.

Upregulation of *ADH* genes (*ADH1A*, *ADH1C*, *ADH5*, and *ADH6*) in tumor tissues was found to be associated with favorable prognosis in HBV-related HCC and may have protective effects in patients receiving hepatic resection. Further studies with a larger sample size are needed to confirm the value of these genes as therapeutic targets in the treatment of HBV-related HCC.

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Disclosure of conflict of interest

None.

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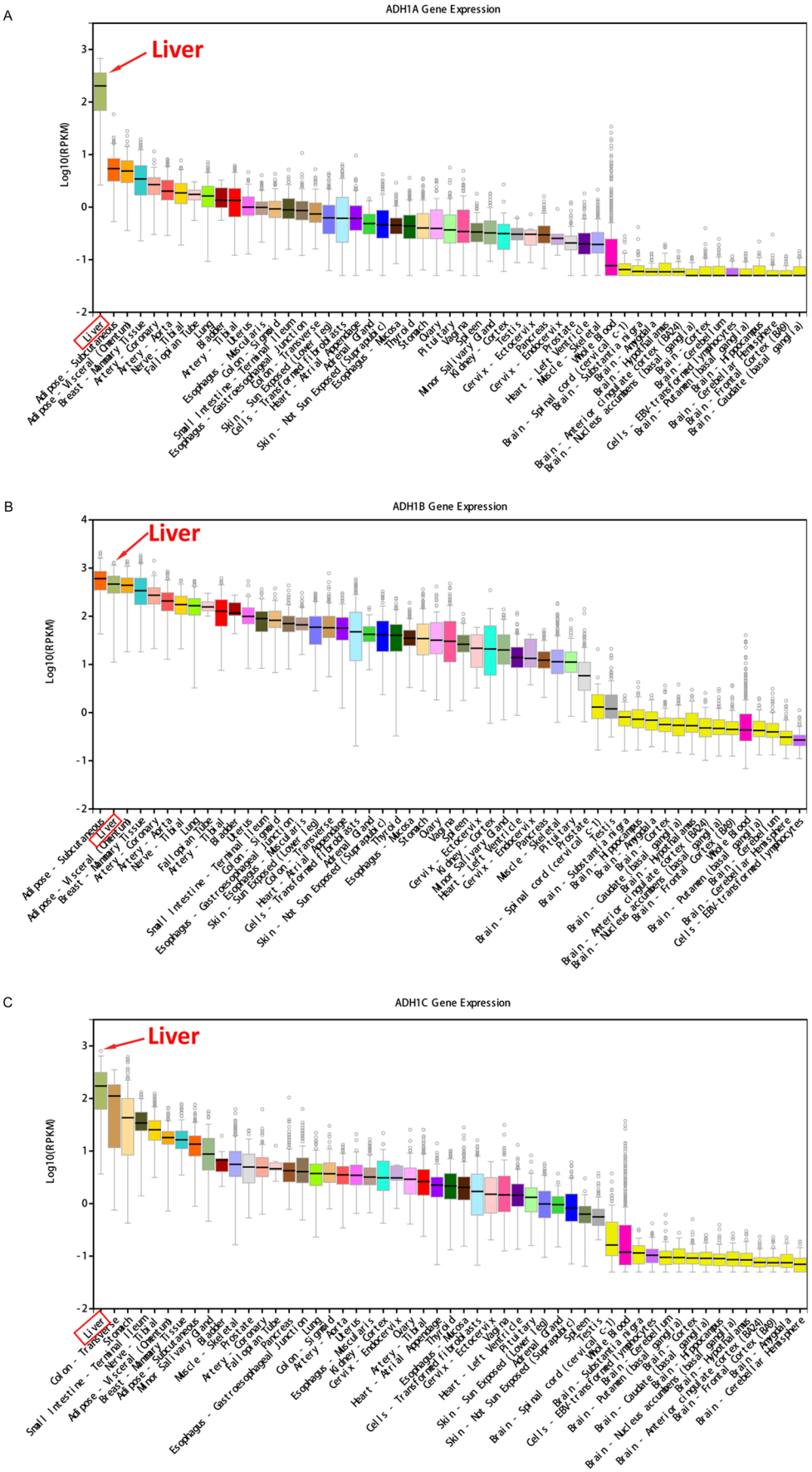
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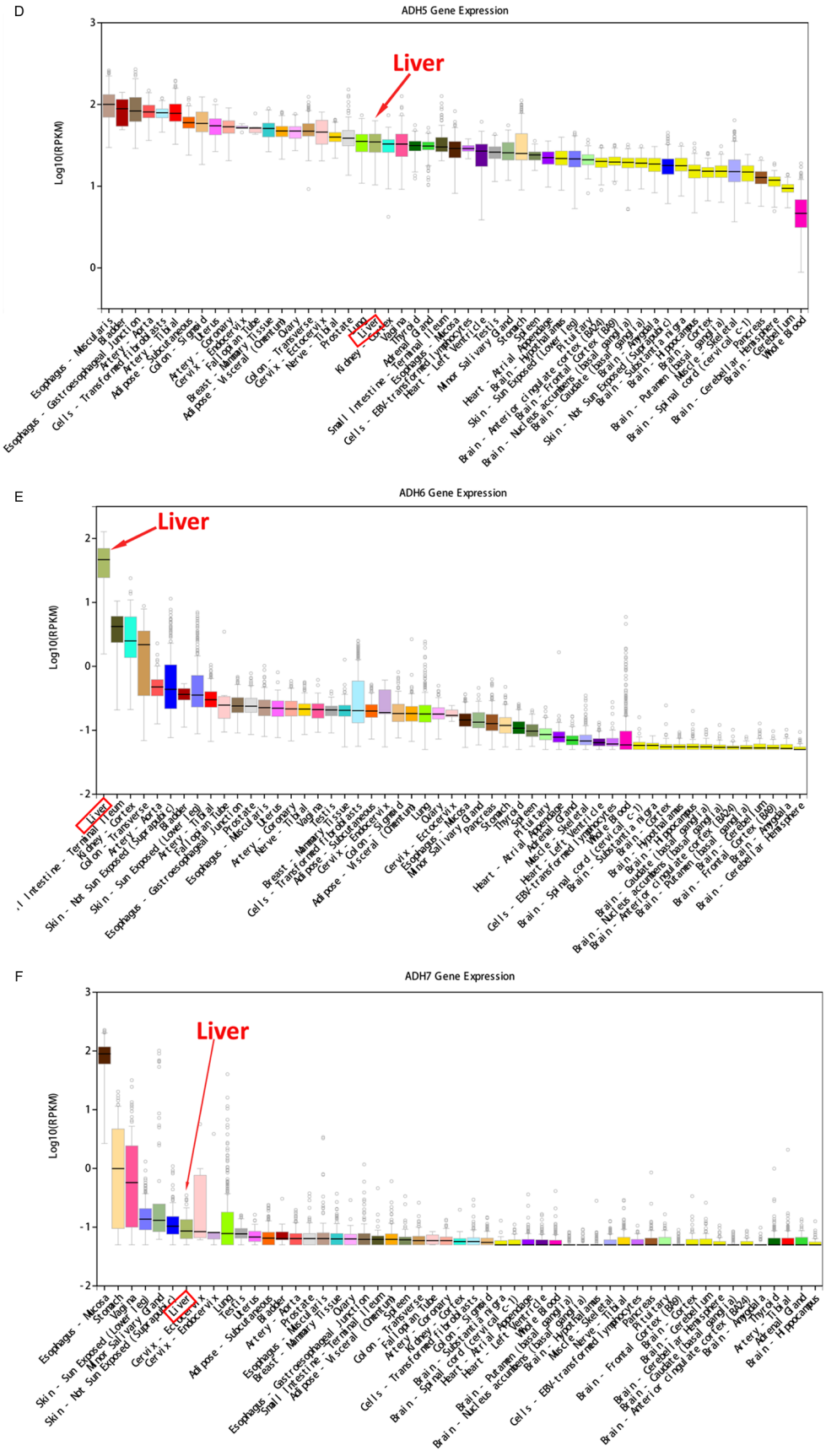
Liver
Log10(RPKM)
B
ADH1B Gene Expression
Liver
Log10(RPKM)
C
ADH1C Gene Expression
Liver
Log10(RPKM)


D
ADH5 Gene Expression
Log10(RPKM)
Liver
E
ADH6 Gene Expression
Log10(RPKM)
Liver
F
ADH7 Gene Expression
Log10(RPKM)
Liver

Supplementary Figure 1. A. ADH1A gene expression in multiple normal tissues. B. ADH1B gene expression in multiple normal tissues. C. ADH1C gene expression in multiple normal tissues. D. ADH5 gene expression in multiple normal tissues. E. ADH6 gene expression in multiple normal tissues. F. ADH7 gene expression in multiple normal tissues.

Supplementary Table 1. Top 20 GSEA enrichment results of ADHA

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
HOSHIDA_LIVER_CANCER_SURVIVAL_DN	104	0.746643	2.030554	P<0.001	0.074579	0.091	1364	Tags = 48%, list = 10%, signal = 53%
PID_HNF3B_PATHWAY	37	0.79728	1.984134	P<0.001	0.074536	0.166	1437	Tags = 57%, list = 11%, signal = 63%
SCHAEFFER_PROSTATE_DEVELOPMENT_AND_CANCER_BOX4_DN	23	0.639044	1.982034	0.002033	0.051743	0.171	1899	Tags = 35%, list = 14%, signal = 40%
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	41	0.834586	1.970976	P<0.001	0.045273	0.193	1641	Tags = 78%, list = 12%, signal = 89%
SHETH_LIVER_CANCER_VS_TXNIP_LOSS_PAM4	198	0.664689	1.946122	P<0.001	0.052568	0.256	863	Tags = 33%, list = 6%, signal = 35%
PID_HNF3A_PATHWAY	38	0.590562	1.945659	P<0.001	0.044381	0.257	1751	Tags = 39%, list = 13%, signal = 45%
COULOUARN_TEMPORAL_TGFB1_SIGNATURE_DN	116	0.630233	1.938828	P<0.001	0.04307	0.287	1730	Tags = 47%, list = 13%, signal = 53%
CAIRO_LIVER_DEVELOPMENT_DN	215	0.720077	1.933612	P<0.001	0.041029	0.301	1446	Tags = 56%, list = 11%, signal = 62%
KEGG_LYSINE_DEGRADATION	38	0.682618	1.919852	P<0.001	0.043401	0.349	1275	Tags = 39%, list = 10%, signal = 44%
CHIANG_LIVER_CANCER_SUBCLASS_UNANNOTATED_UP	58	0.816577	1.919385	P<0.001	0.039271	0.351	1361	Tags = 62%, list = 10%, signal = 69%
ACEVEDO_LIVER_CANCER_DN	397	0.675502	1.905973	P<0.001	0.043596	0.398	1671	Tags = 48%, list = 13%, signal = 54%
BOCHKIS_FOXA2_TARGETS	320	0.559642	1.902829	P<0.001	0.041826	0.41	1753	Tags = 35%, list = 13%, signal = 40%
ACEVEDO_NORMAL_TISSUE_ADJACENT_TO_LIVER_TUMOR_DN	256	0.589372	1.900327	0.001942	0.040281	0.422	1820	Tags = 36%, list = 14%, signal = 41%
SERVITJA_ISLET_HNF1A_TARGETS_DN	77	0.656267	1.900026	P<0.001	0.037596	0.422	1348	Tags = 45%, list = 10%, signal = 50%
IIZUKA_LIVER_CANCER_PROGRESSION_G2_G3_UP	24	0.822648	1.89814	P<0.001	0.035924	0.43	2145	Tags = 83%, list = 16%, signal = 99%
LEE_LIVER_CANCER_ACOX1_DN	62	0.795563	1.894656	P<0.001	0.034857	0.44	1170	Tags = 53%, list = 9%, signal = 58%
REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES	169	0.639955	1.890827	P<0.001	0.034863	0.452	1163	Tags = 35%, list = 9%, signal = 38%
SOTIRIOU_BREAST_CANCER_GRADE_1_VS_3_DN	39	0.685199	1.879148	P<0.001	0.039175	0.515	2368	Tags = 59%, list = 18%, signal = 72%
KEGG_FATTY_ACID_METABOLISM	35	0.853894	1.871046	P<0.001	0.041684	0.544	1311	Tags = 80%, list = 10%, signal = 88%
KEGG_PROPANOATE_METABOLISM	27	0.806565	1.867816	P<0.001	0.041072	0.558	1360	Tags = 70%, list = 10%, signal = 78%

Supplementary Table 2. Top 20 GSEA enrichment results of ADH1C

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
CAIRO_HEPATOBLASTOMA_CLASSES_DN	187	0.802441	1.956468	P<0.001	0.415669	0.226	1550	Tags = 67%, list = 12%, signal = 75%
BURTON_ADIPOGENESIS_6	158	0.595723	1.94419	P<0.001	0.249218	0.257	1998	Tags = 42%, list = 15%, signal = 49%
HOUSTIS_ROS	34	0.770789	1.934926	P<0.001	0.1938	0.291	2026	Tags = 59%, list = 15%, signal = 69%
KEGG_TYROSINE_METABOLISM	35	0.763226	1.916672	P<0.001	0.18821	0.347	1524	Tags = 40%, list = 11%, signal = 45%
KEGG_TRYPTOPHAN_METABOLISM	32	0.846824	1.914434	P<0.001	0.15404	0.352	1251	Tags = 69%, list = 9%, signal = 76%
HOSHIDA_LIVER_CANCER_SUBCLASS_S3	248	0.837974	1.90638	P<0.001	0.145274	0.38	1314	Tags = 74%, list = 10%, signal = 81%
KEGG_BUTANOATE_METABOLISM	31	0.774436	1.904073	P<0.001	0.128246	0.383	1031	Tags = 55%, list = 8%, signal = 59%
REACTOME_BIOLOGICAL_OXIDATIONS	109	0.7657	1.902508	P<0.001	0.115003	0.389	1345	Tags = 59%, list = 10%, signal = 65%
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	52	0.859774	1.89029	P<0.001	0.122347	0.441	1374	Tags = 73%, list = 10%, signal = 81%
REACTOME_PHASE_II_CONJUGATION	48	0.785464	1.882603	P<0.001	0.121892	0.466	1273	Tags = 60%, list = 10%, signal = 67%
ACEVEDO_LIVER_CANCER_DN	397	0.660644	1.87092	P<0.001	0.129507	0.508	2059	Tags = 50%, list = 15%, signal = 58%
SPIRA_SMOKERS_LUNG_CANCER_UP	35	0.758693	1.850824	P<0.001	0.156581	0.582	656	Tags = 29%, list = 5%, signal = 30%
CHIANG_LIVER_CANCER_SUBCLASS_UNANNOTATED_UP	58	0.78767	1.843463	P<0.001	0.15926	0.608	1329	Tags = 59%, list = 10%, signal = 65%
BOYAULT_LIVER_CANCER_SUBCLASS_G1_DN	36	0.876597	1.84159	P<0.001	0.151747	0.615	1590	Tags = 94%, list = 12%, signal = 107%
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	64	0.79062	1.834657	P<0.001	0.155165	0.64	1596	Tags = 63%, list = 12%, signal = 71%
TSUNODA_CISPLATIN_RESISTANCE_DN	44	0.676448	1.831794	P<0.001	0.151094	0.648	1603	Tags = 50%, list = 12%, signal = 57%
KEGG_HISTIDINE_METABOLISM	22	0.753428	1.828263	0.001984	0.149407	0.661	1638	Tags = 59%, list = 12%, signal = 67%
KEGG_RETINOL_METABOLISM	43	0.851498	1.822884	P<0.001	0.150132	0.677	1209	Tags = 72%, list = 9%, signal = 79%
SHETH_LIVER_CANCER_VS_TXNIP_LOSS_PAM4	198	0.625686	1.821781	P<0.001	0.144713	0.682	1007	Tags = 36%, list = 8%, signal = 38%
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	55	0.854561	1.82021	P<0.001	0.140282	0.692	1374	Tags = 76%, list = 10%, signal = 85%

Supplementary Table 3. Top 20 GSEA enrichment results of ADH5

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
SHEN_SMARCA2_TARGETS_UP	347	0.614099	1.96963	P<0.001	0.267056	0.2	3486	Tags = 64%, list = 26%, signal = 85%
FLECHNER_BIOPSY_KIDNEY_TRANSPLANT_REJECTED_VS_OK_DN	490	0.60512	1.95085	P<0.001	0.171147	0.24	2738	Tags = 58%, list = 21%, signal = 70%
MOOTHA_MITOCHONDRIA	418	0.511119	1.863074	0.003976	0.356493	0.539	2857	Tags = 44%, list = 21%, signal = 55%
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	41	0.780803	1.840249	P<0.001	0.363532	0.628	2064	Tags = 78%, list = 15%, signal = 92%
MOOTHA_HUMAN_MITODB_6_2002	407	0.514765	1.830548	0.005941	0.328087	0.655	2871	Tags = 45%, list = 22%, signal = 56%
ACEVEDO_NORMAL_TISSUE_ADJACENT_TO_LIVER_TUMOR_DN	256	0.553115	1.789675	0.003929	0.469904	0.787	2844	Tags = 47%, list = 21%, signal = 58%
KEGG_PROPANOATE_METABOLISM	27	0.758891	1.783667	0.006224	0.435957	0.806	1014	Tags = 59%, list = 8%, signal = 64%
UEDA_PERIFERAL_CLOCK	123	0.560324	1.775862	P<0.001	0.419906	0.832	2723	Tags = 49%, list = 20%, signal = 61%
HOSHIDA_LIVER_CANCER_SURVIVAL_DN	104	0.644922	1.758561	0.002004	0.461178	0.87	1819	Tags = 47%, list = 14%, signal = 54%
LUCAS_HNF4A_TARGETS_UP	52	0.669112	1.753128	0.004115	0.440649	0.882	1854	Tags = 50%, list = 14%, signal = 58%
KEGG_TYROSINE_METABOLISM	35	0.681066	1.723439	0.002092	0.562175	0.935	982	Tags = 40%, list = 7%, signal = 43%
KEGG_PEROXISOME	67	0.719978	1.71696	0.00818	0.555119	0.948	2868	Tags = 75%, list = 22%, signal = 95%
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	16	0.774674	1.711986	0.002066	0.54221	0.95	1043	Tags = 63%, list = 8%, signal = 68%
KEGG_BETA_ALANINE_METABOLISM	21	0.71657	1.709855	0.010288	0.516777	0.95	2005	Tags = 67%, list = 15%, signal = 78%
SHETH_LIVER_CANCER_VS_TXNIP_LOSS_PAM4	198	0.576228	1.708068	0.008197	0.491595	0.951	2218	Tags = 43%, list = 17%, signal = 51%
WONG_MITOCHONDRIA_GENE_MODULE	184	0.464797	1.704497	0.055227	0.479663	0.957	3682	Tags = 53%, list = 28%, signal = 72%
KEGG_HISTIDINE_METABOLISM	22	0.682512	1.697962	0.0125	0.482191	0.965	2365	Tags = 64%, list = 18%, signal = 77%
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	27	0.835804	1.697437	0.007797	0.457934	0.965	1302	Tags = 74%, list = 10%, signal = 82%
WAKABAYASHI_ADIPOGENESIS_PPARG_RXRA_BOUND_WITH_H4K20ME1_MARK	104	0.496037	1.696148	0.008016	0.439943	0.967	2218	Tags = 39%, list = 17%, signal = 47%
WOO_LIVER_CANCER_RECURRENCE_DN	75	0.845553	1.695638	0.001992	0.420547	0.967	1221	Tags = 84%, list = 9%, signal = 92%

Supplementary Table 4. Top 20 GSEA enrichment results of ADH6

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
MOOTHA_MITOCHONDRIA	418	0.57029	2.028491	P<0.001	0.098193	0.086	2561	Tags = 43%, list = 19%, signal = 51%
COULOUARN_TEMPORAL_TGFB1_SIGNATURE_DN	116	0.656916	2.01586	P<0.001	0.057755	0.099	1822	Tags = 48%, list = 14%, signal = 55%
HOSHIDA_LIVER_CANCER_SURVIVAL_DN	104	0.736942	2.003667	P<0.001	0.04521	0.115	1776	Tags = 53%, list = 13%, signal = 61%
BOCHKIS_FOXA2_TARGETS	320	0.58259	1.981637	P<0.001	0.049893	0.152	1899	Tags = 37%, list = 14%, signal = 42%
FLECHNER_BIOPSY_KIDNEY_TRANSPLANT_REJECTED_VS_OK_DN	490	0.610343	1.961969	P<0.001	0.052266	0.193	1681	Tags = 40%, list = 13%, signal = 44%
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	41	0.852069	1.956236	P<0.001	0.048219	0.21	1578	Tags = 80%, list = 12%, signal = 91%
MOOTHA_HUMAN_MITODB_6_2002	407	0.558954	1.936948	0.002105	0.056014	0.273	2561	Tags = 44%, list = 19%, signal = 53%
ACEVEDO_NORMAL_TISSUE_ADJACENT_TO_LIVER_TUMOR_DN	256	0.596508	1.92055	P<0.001	0.06201	0.318	1873	Tags = 38%, list = 14%, signal = 43%
KEGG_LYSINE_DEGRADATION	38	0.672505	1.90313	P<0.001	0.069656	0.367	1672	Tags = 45%, list = 13%, signal = 51%
HOSHIDA_LIVER_CANCER_LATE_RECURRENCE_DN	65	0.622985	1.901125	P<0.001	0.064096	0.374	848	Tags = 34%, list = 6%, signal = 36%
HOSHIDA_LIVER_CANCER_SUBCLASS_S3	248	0.845669	1.900867	P<0.001	0.058565	0.375	1219	Tags = 74%, list = 9%, signal = 80%
KEGG_TYROSINE_METABOLISM	35	0.775984	1.898256	P<0.001	0.055739	0.389	903	Tags = 40%, list = 7%, signal = 43%
SHETH_LIVER_CANCER_VS_TXNIP_LOSS_PAM4	198	0.650788	1.897512	P<0.001	0.052372	0.395	1374	Tags = 38%, list = 10%, signal = 42%
KEGG_PROPANOATE_METABOLISM	27	0.835786	1.893706	P<0.001	0.051449	0.409	1517	Tags = 74%, list = 11%, signal = 83%
REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES	169	0.642203	1.88226	0.001946	0.056409	0.447	1187	Tags = 37%, list = 9%, signal = 40%
ACEVEDO_LIVER_CANCER_DN	397	0.659783	1.880445	0.001965	0.053874	0.449	1810	Tags = 49%, list = 14%, signal = 55%
REACTOME_BIOLOGICAL_OXIDATIONS	109	0.7766	1.879382	P<0.001	0.051521	0.453	849	Tags = 50%, list = 6%, signal = 53%
KEGG_PHENYLALANINE_METABOLISM	17	0.824741	1.878918	P<0.001	0.04905	0.456	510	Tags = 47%, list = 4%, signal = 49%
RODWELL_AGING_KIDNEY_DN	110	0.554667	1.875812	P<0.001	0.048975	0.473	1499	Tags = 33%, list = 11%, signal = 37%
KEGG_HISTIDINE_METABOLISM	22	0.775955	1.873437	P<0.001	0.04826	0.483	1632	Tags = 68%, list = 12%, signal = 78%