ALDH1L1 variant rs2276724 and mRNA expression predict post-operative clinical outcomes and are associated with *TP53* expression in HBV-related hepatocellular carcinoma

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Abstract. Aldehyde dehydrogenase 1 family member L1 (ALDH1L1) is downregulated in hepatocellular carcinoma (HCC) tumors, and its decreased expression is associated with the poor prognosis of HCC patients. We, therefore, evaluated the effect of single nucleotide polymorphisms (SNPs) of *ALDH1L1*, and its mRNA expression on the survival of hepatitis B virus (HBV)-related HCC patients and the association with tumor protein p53 (TP53) expression. *ALDH1L1* SNPs in 415 HBV-related HCC patients were genotyped via direct sequencing. Expression profile chip datasets and survival information were obtained from GSE14520. The C allele (CT/CC) carriers of rs2276724 were significantly associated with a favorable prognosis [adjusted P=0.040; adjusted hazard ratio (HR)=0.725; 95% confidence interval (CI)=0.533-0.986]. Joint-effect analyses suggested that the CT/CC genotype of rs2276724 in TP53-negative patients was significantly associated with a decreased risk of death, compared to the TT genotype of rs2276724 in TP53-positive patients (adjusted P=0.037; adjusted HR=0.621; 95% CI=0.396-0.973). Furthermore, low expression of *ALDH1L1* predicted a poor prognosis for the HBV-related HCC patients (adjusted P=0.04 for disease-free survival; adjusted P=0.001 for overall survival). Patients with high *ALDH1L1* expression and low *TP53* expression were significantly associated with a decreased risk of recurrence and death, and patients with a high *TP53* expression were also significantly associated with a decreased risk of death in HBV-related HCC, compared with low *ALDH1L1* and low *TP53* expression. Our results suggest that ALDH1L1 may be a biomarker for predicting postoperative clinical outcomes. Moreover, *ALDH1L1-rs2276724* and mRNA expression were associated with *TP53 expression* in HBV-related HCC patients.

Introduction

Liver cancer is the second leading cause of cancer-related deaths in males worldwide. More than half of these liver cancer-related deaths occurred in China during 2012 (1). A recent study estimated that approximately 422,100 Chinese patients died from liver cancer in 2015, which will make it the third leading cause of cancer-related death in China (2). A population-based study of 138,852 cancer cases reported that liver cancer is associated with poor survival with an age-standardized 5-year relative survival of 10.1% in China (3). Liver cancer death rates in Guangxi Province were the highest in China for both males and females (4). Hepatocellular carcinoma (HCC) is the most common type of liver cancer (85-90%) (5). The most prominent parameters associated with HCC in China include hepatitis B virus (HBV) and C viral infection, alcoholic liver disease, and aflatoxin-B1-contaminated food (6). Previous studies of the Guangxi population reported that high HBV infection and aflatoxin B1 (AFB1)

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exposure resulted in a higher HCC morbidity and mortality in this province than in other provinces in China (7,8).

Tumor protein p53 (TP53) is a tumor-suppressor protein involved in transcriptional activation, DNA binding, and oligomerization domains. TP53 wild-type protein can induce cell cycle arrest, apoptosis, senescence, DNA repair, and changes in metabolism (9,10). Wild-type TP53 is an important tumor-suppressor gene in many types of cancers, especially in HCC, and its mutation is regarded as oncogenic (10,11), and affects HCC tumorigenesis and cancer progression (12-16). AFB1 has been strongly associated with TP53 mutations at codon 249 in exon 7, and HCC patients in Guangxi have a high rate (34%) of TP53 mutations at codon 249 in exon 7 (8,17). Thus, the population in this region presents a unique opportunity to investigate the relationship of HBV infection, AFB1 exposure, and TP53 gene mutations with HCC. Recently, meta-analyses have reported that immunohistochemical characterization of TP53 expression is associated with a poor prognosis of HCC (18).

Aldehyde dehydrogenase 1 family member L1 (ALDH1L1), also known as 10-formyltetrahydrofolate dehydrogenase (FDH), is a folate metabolism enzyme with tumor suppressor-like properties and is involved in the regulation of cell proliferation. A previous study conducted by Oleinik and Krupenko demonstrated that the antiproliferative effects of FDH in human lung cancer cell line A549 induced G1 arrest and apoptosis, accompanied by an increase in TP53 and p21 (19). In addition, subsequent research by this group also demonstrated that FDH-induced tumor-suppressor effects were strictly TP53-dependent in A549 cells and the TP53 pathway was a downstream mechanism in response to induction of FDH expression (20). Another study of HCC patients in Guangxi reported that low ALDH1L1 protein expression was a new and potential prognostic marker for the survival of HCC patients (21). Using bioinformatic analyses, we found that expression of ALDH1L1 in the liver was the highest in various human normal tissues, and was significantly downregulated in HCC tumor tissues compared to tissues adjacent to the tumor. Our previous genome-wide association study also reported that single nucleotide polymorphisms (SNPs) were associated with positive immunohistochemical characterization of TP53 expression in Guangxi patients with HBV-related HCC (22). In the present study, we determined the association between ALDH1L1 genetic variations and mRNA expression and the postoperative prognosis in Chinese HBV-related HCC patients, and its interaction with TP53.

Materials and methods

Study population. This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Guangxi, China) with approval number KY-E-032. Fresh specimens of 415 cases of HCC were collected from 2001 to 2013 at the First Affiliated Hospital of Guangxi Medical University and were confirmed by pathology. All the patients were positive for serum HBV surface antigen inspection. The TP53 expression status in the cancer tissues was detected by immunohistochemistry. The cancer tissues were collected during surgery and immediately stored at -80°C for further use. The tumor status was classified using the Barcelona Clinic Liver Cancer (BCLC) staging system, and the liver reserve function was determined using the Child-Pugh classification. Portal vein tumor thrombus (PVTT) was classified according to a previous study (23).

SNP selection and genotyping. ALDH1L1 tagged SNPs and non-synonymous SNPs were selected by using an SNP info Web Server (http://snpinfo.niehs.nih.gov/. accessed 20 October 2016). Evaluation of SNP non-synonymous mutations caused by changes in protein amino acids were determined using SIFT (http://sift.jcvi.org/. accessed October 20, 2016) (24) and PolPhen 2 (http://genetics.bwh.harvard.edu/pph2/index.shtml. accessed October 20, 2016) (25). Bioinformatic analyses of ALDH1L1 SNPs with the tagged SNP located in the exon region showed that rs2276724 was a non-synonymous SNP. The influence of SNP non-synonymous mutation analyses by SIFT also showed that rs2276724 S481G was deleterious to protein coding. Consistent results from PolyPhen2 also showed that rs2276724 S481G was possibly deleterious to protein coding. Thus, rs2276724 was further studied. The transcriptional regulation of rs2276724 S481G was detected by F-SNP database (http://compbio.cs.queensu.ca/F-SNP/. accessed February 7, 2017) and the prediction tool Golden Path suggested that non-synonymous mutation of rs2276724 S481G caused transcriptional regulation change (26).

Genomic DNA was extracted from surgical tumor samples using the TIANamp Genomic DNA kit (Tiangen Biotech, Beijing, China). All samples were genotyped by DNA sequencing using an ABI Prism 3100 (Applied Biosystems, Shanghai Sangon Biological Engineering Technology and Services, Shanghai, China) with the following primers: forward, 5'-GCCCTGTCTTCCCTTCCTGTG-3' and reverse, 5'-CCTGAGCCCACTCTGCTGAAAT-3' for rs2276724. The sequencing results were analyzed using Chromas software (http://technelysium.com.au/wp/chromas/ accessed October 20, 2016) with a signal/noise >98%.

GEO data and bioinformatic analysis. Based on the predictive result of F-SNP, non-synonymous mutation of rs2276724 S481G may affect gene transcriptional regulation. We hypothesized that ALDH1L1 mRNA expression may contribute to prognostic prediction of HBV-related HCC. To test this hypothesis, we further analyzed the association of ALDH1L1 and TP53 at the transcriptional level to evaluate the effects of ALDH1L1 mRNA expression and the interactions with TP53 on HCC prognosis after hepatectomy. The profile chip dataset of Chinese HBV-related HCC from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/. accessed October 20, 2016) was analyzed and Spearman's correlation coefficient was used to assess its correlations. The GEO data selection criteria were set as follows: i), expression profiling chip; ii), Chinese HBV-related HCC; iii), corresponding survival profiles was available; and iv), patients undergoing hepatectomy. By searching the GEO database, we found that only the data of GSE14520 met the criteria above. Then, the samples were divided into two groups according to the ALDH1L1 expression in tumors. The high ALDH1L1 group was composed of samples with ALDH1L1 expression levels above the median value, and the low ALDH1L1 group was composed of the remaining samples. TP53 expression was

grouped in the same manner. Both disease-free survival (DFS) and overall survival (OS) were analyzed in the different *ALDH1L1* expression groups and used for the joint-effect survival analyses of the *TP53* groups. We also stratified the analyses of associations between different *ALDH1L1* expression levels and clinical features, both for OS and DFS. A gene interaction analysis web site (GeneMANIA: http://www.genemania.org/ accessed October 20, 2016) was used for correlation analyses between genes. Online analysis tool was used to analyze the *ALDH1L1* expression in multiple human normal tissues (http://www.gtexportal.org/home/ accessed October 20, 2016) and in differences of expression of HCC tumor and adjacent tumor tissues (MERAV, Metabolic gEne Rapid Visualizer :http://merav.wi.mit.edu/. accessed October 20, 2016).

Statistical analysis. Hardy-Weinberg equilibrium (HWE) of the selected SNPs was estimated using a goodness-of-fit χ^2-test. The binary logistic regression model was used to analyze the genetic model of *ALDH1L1* genotypes for the status of different *TP53* expression levels and for the association between clinical risk factors with *ALDH1L1* genotypes. Survival analyses were performed using the Kaplan-Meier method with the log-rank test for different clinical factors and different genotypes. Cox proportion haphazard regression analyses were used to calculate the crude or adjusted hazard ratio (HR) and the 95% confidence interval (CI) in univariate analyses and multivariate analyses, adjusted for those variables with $P<0.1$ in later multivariate analyses. A value of $P<0.05$ was considered statistically significant. All statistical analyses were conducted using SPSS Statistical Software for Windows, version 20.0 (SPSS, Chicago, IL, USA).

Results

Clinical features and outcomes. Patients were followed up after surgery until the final follow-up or until death. The final follow-up was conducted in September 2014. A total of 415 patients successfully completed the follow-up, with 6.7% of the patients lost in the follow-up. The duration of the follow-up ranged from 12-125 months, with an overall median survival time (MST) of 48 months. At the time of analyses, 192 (46.3%) of the patients had died. A total of 162 patients were negative for *TP53* expression and 253 patients were positive for *TP53* expression. Clinical features of all patients and the association with the OS are shown in Table I. Using Kaplan-Meier analyses, the biological characteristics of tumor size, tumor number, BCLC stage, and portal vein tumor thrombus (PVTT) were significantly associated with the OS (log-rank test, P<0.001) and increased risk of death. A Child-Pugh classification score for 356 (85.8%) patients was associated with the OS (log-rank P=0.005). Radical resection was conducted in 231 patients (55.7%), and was associated with the OS (log-rank P=0.052), and patients without cirrhosis had a better prognosis (log-rank P=0.027). Adjuvant antiviral treatment in 143 (34.5%) patients was significantly associated with the OS (log-rank P=0.019), compared with those without treatment. The other clinical parameters were not associated with the OS.

Gene expression analysis. Bioinformatic analysis of *ALDH1L1* gene expression in multiple human normal tissues showed that *ALDH1L1* was the highest expression in normal liver tissue (Fig. 1A). *ALDH1L1* expression was significantly downregulated in HCC tumor tissue, as determined for MERAV (Fig. 1B) and GSE14520 (Fig. 1C).

Genetic model analysis of rs2276724. The success of genotyping for rs2276724 was 100%. The genotype frequencies met Hardy-Weinberg equilibrium as shown by the goodness-of-fit χ^2-test (rs2276724, χ^2=0.236; P=0.627). The genotype distribution of rs2276724 in patients with different *TP53* expression is shown in Table II. The binary logistic regression model was used for adjustment for alcohol consumption, the Child-Pugh score, tumor size, tumor number, BCLC stage, radical resection, cirrhosis, adjuvant antiviral treatment, and PVTT. Using a co-dominant genetic model, the CT genotype of rs2276724 was significantly reduced with *TP53* expression in HBV-related HCC patients (adjusted P=0.045; adjusted OR=0.644; 95% CI=0.418-0.990), compared with the TT genotype. The genotype distributions of rs2276724 in different types of *TP53* expression were similar to the four genetic models.

Genetic polymorphisms in the HCC risk factors and in stratified analysis. Association between risk factors and rs2276724 genotypes are summarized in Table III. None of the selected risk factors was associated with rs2276724 genotypes in the present study. Stratified analyses of the rs2276724 genotype and different strata of the OS of selected risk factors are shown in Fig. 2. In the favorable strata, the CT/CC genotype of rs2276724 significantly decreased the risk of death among patients with tumor sizes ≤5 cm, Child-Pugh score A, and without a PVTT. Regarding the invasion in the adverse strata, we also observed a similar effect that the CT/CC genotype of rs2276724 significantly decreased the risk of death among patients with BCLC stage B/C, non-radical resection, and the presence of regional invasion. The genotype distributions of rs2276724 in other strata showed no difference.

Relationship of rs2276724 and TP53 status with the OS. Using a dominant genetic model, patients with the TT genotype had a shorter MST compared to those with CT or CC genotypes of rs9275572 (TT vs. CT vs. CC; 39 vs. 50 vs. 79 months; log-rank P=0.017; Fig. 3A). However, the difference was similar after adjustment for alcohol consumption, Child-Pugh score, tumor size, tumor number, BCLC stage, radical resection, cirrhosis, adjuvant antiviral treatment, and a PVTT. In the dominant genetic model, patients with the TT genotype had a significantly smaller MST than the C allele carriers (TT vs. CT/CC; 39 vs. 58 months; log-rank P=0.009; Fig. 3B), and the CT/CC genotype of rs2276724 had a significantly decreased risk of death (adjusted P=0.040; adjusted HR=0.725; 95% CI=0.533-0.986; Table IV). Haplotype analysis showed that the C allele was associated with a significantly decreased risk of death (adjusted P=0.032; adjusted HR=0.747; 95% CI=0.572-0.976; Table IV), compared with the T allele. The prognosis for a different status of *TP53* expression was similar in the patients (adjusted P=0.280; adjusted HR=1.183; 95% CI=0.872-1.605; Table IV and Fig. 3C).

Table I. Clinical features of the patients with HBV-related HCC.

Variables	Patients (N=415)	No. of events (%)	MST (months)	HR (95% CI)	Log-rank p-value
Age (years)					0.149
≤60	367	169 (46.0)	51	1	
>60	48	23 (47.9)	41	1.375 (0.888-2.128)	
Sex					0.479
Male	375	177 (47.2)	48	1	
Female	40	15 (37.5)	42	0.828 (0.488-1.404)	
Ethnicity					0.989
Han	260	122 (46.9)	47	1	
Minority	155	70 (45..2)	50	0.998 (0.743-1.341)	
BMI					0.745
≤25	328	150 (45.7)	45	1	
>25	87	42 (48.3)	51	0.945 (0.6670-1.333)	
Smoking status					0.107
None	263	118 (44.9)	51	1	
Ever	152	74 (48.7)	39	1.269 (0.947-1.702)	
Drinking status					0.084
None	246	107 (43.5)	51	1	
Ever	169	85 (50.3)	40	1.284 (0.964-1.710)	
Child-Pugh score					0.005
A	356	153 (43.0)	51	1	
B	59	39 (66.1)	31	1.689 (1.159-2.460)	
Cirrhosis					0.027
No	46	16 (34.8)	NA	1	
Yes	369	176 (47.7)	44	1.769 (1.058-2.958)	
Radical resection[a]					0.052
Yes	231	97 (42.0)	71	1	
None	172	89 (51.7)	40	1.330 (0.997-1.774)	
Portal hypertension[b]					0.243
No	208	100 (48.1)	52	1	
Yes	172	75 (43.6)	42	1.197 (0.883-1.623)	
Pathological diagnosis[c]					0.622
Well differentiated	24	9 (37.5)	79	1	
Moderately differentiated	341	159 (46.6)	44	1.378 (0.703-2.699)	
Poorly differentiated	11	4 (36.4)	NA	1.200 (0.369-3.898)	
Adjuvant antiviral treatment				0.019	
Yes	143	43 (30.0)	NA	1	
No	272	149 (54.8)	41	1.501 (1.065-2.116)	
AFP level[d]					0.233
<400	210	89 (42.4)	51	1	
≥400	175	85 (48.6)	42	1.197 (0.889-1.612)	
Tumor behavior					
Tumor size (cm)					<0.001
≤5	158	59 (37.3)	75	1	
>5	229	133 (58.1)	36	1.802 (1.326-2.450)	
Tumor number					<0.001
Single	302	127 (42.0)	58	1	
Multiple	113	65 (57.5)	28	1.792 (1.326-2.420)	
Regional invasion					0.156
Absence	353	162 (45.9)	51	1	
Presence	62	30 (48.4)	35	1.323 (0.895-1.958)	

Table I. Continued.

Variables	Patients (N=415)	No. of events (%)	MST (months)	HR (95% CI)	Log-rank p-value
BCLC stage					<0.001
A	236	81 (34.3)	95	1	
B	68	37 (54.4)	36	2.055 (1.391-3.035)	
C	111	74 (66.7)	24	2.741 (1.994-3.767)	
PVTT					<0.001
No	342	139 (40.6)	73	1	
Yes	73	53 (72.6)	18	2.801 (2.032-3.861)	

[a]Information regarding radical resection was unavailable for 12 patients; [b]information regarding portal hypertension was unavailable for 35 patients; [c]information regarding pathological diagnosis was unavailable for 39 patients; [d]information regarding AFP level was unavailable for 30 patients. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; BMI, body mass index; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; PVTT, portal vein tumor thrombus; MST, median survival time; HR, hazard ratio; CI, confidence interval.
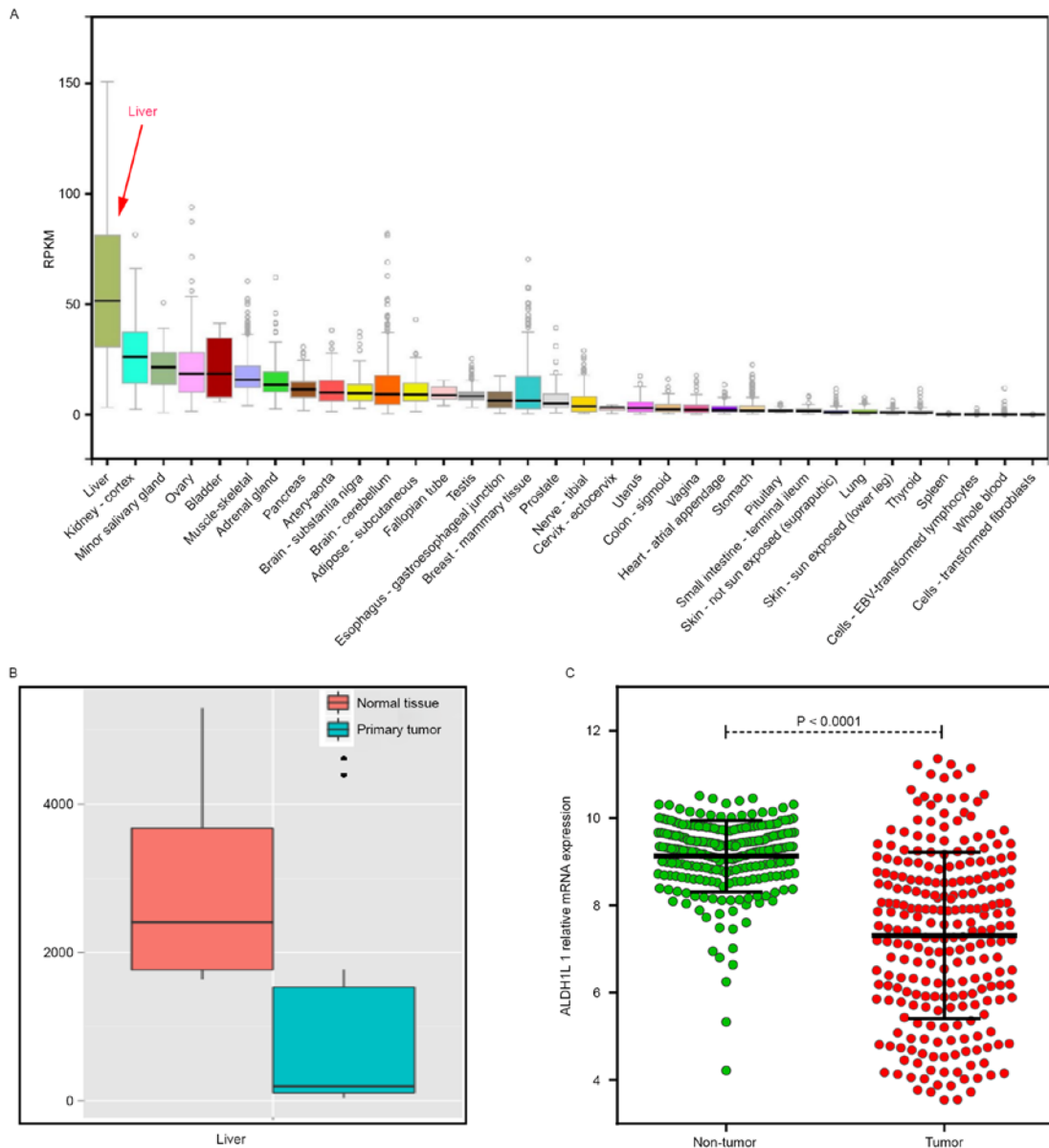

Figure 1. (A) *ALDH1L1* gene expression in multiple normal tissues. (B) Comparison of *ALDH1L1* expression between HCC and normal tissues by MERAV. (C) Comparison of *ALDH1L1* expression between HCC and non-tumor tissues by GSE14520. ALDH1L1, aldehyde dehydrogenase 1 family member L1; HCC, hepatocellular carcinoma.

Table II. Genotype distribution of rs2276724 in HBV-related HCC patients with different TP53 expression statuses (genetic model).

SNP	TP53-negative (n=162)	TP53-positive (n=253)	Crude OR (95% CI)	Crude p-value	Adjusted OR (95% CI)	Adjusted P-value[a]
rs2276724						
Allele						
T	246	394	1		1	
C	78	112	0.894 (0.640-1.249)	0.512	0.878 (0.623-1.238)	0.458
Co-dominant						
TT	89	156	1		1	
CT	68	82	0.688 (0.455-1.040)	0.076	0.644 (0.418-0.990)	0.045
CC	5	15	1.712 (0.602-4.867)	0.314	1.838 (0.629-5.365)	0.266
Dominant						
TT	89	156	1		1	
CT+CC	73	97	0.758 (0.508-1.131)	0.175	0.725 (0.479-1.097)	0.128
Recessive						
CT+TT	157	238	1		1	
CC	5	15	1.979 (0.705-5.554)	0.195	2.153 (0.747-6.207)	0.156

[a]Adjustment for drinking status, Child-Pugh score, tumor size, tumor number, BCLC stage, radical resection, cirrhosis, adjuvant antiviral treatment, PVTT in logistic regression model. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TP53, tumor protein p53; SNP, single nucleotide polymorphism; AFP, α-fetoprotein; OR, odds ratio; CI, confidence interval.
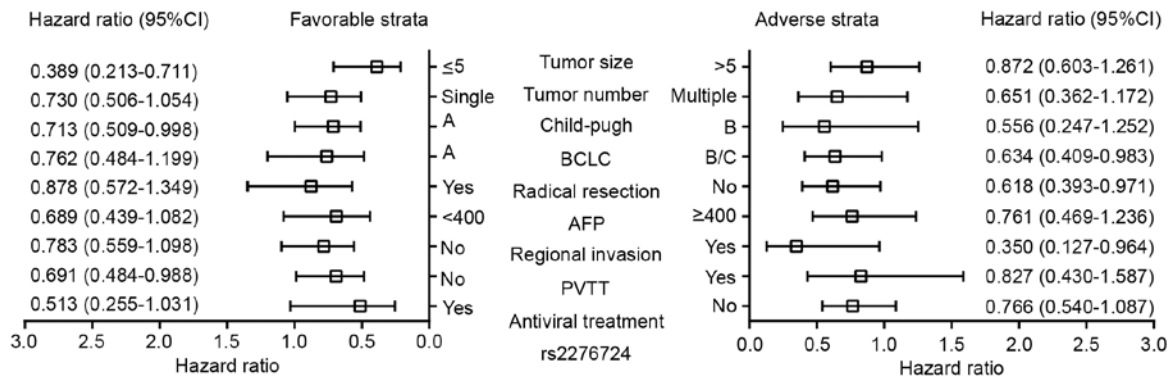

Figure 2. Stratified analyses of associations of rs2276724 with the OS in the patients with HBV-related HCC. All variables were stratified by favorable and adverse strata. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OS, overall survival.

Joint-effect analysis. We further analyzed the TP53 status and rs2276724 genotypes with the mutual association with OS of the HBV-related HCC patients. TP53-positive patients with CT/CC genotypes had a significantly longer MST (Table V and Fig. 3D) as compared to the TP53-positive patients with TT genotype. After adjustment for alcohol consumption, Child-Pugh score, tumor size, tumor number, BCLC stage, radical resection, cirrhosis, adjuvant antiviral treatment, PVTT in the Cox proportion haphazard regression model, the TP53-negative patients with CT/CC genotypes showed a significantly decreased risk of death (adjusted P=0.037; adjusted HR=0.621; 95% CI=0.396-0.973; Table V).

GEO data and gene interaction analysis. In order to determine the relationship of *ALDH1L1* with *TP53* at the transcriptional level, the GSE14520 database (including 218 Chinese HBV-related HCC patients with clinical information and prognosis) was used to correlate the *ALDH1L1* and *TP53* mRNA expression in HBV-related HCC patients. The results showed that *ALDH1L1* had a weak negative correlation with *TP53* (r=-0.396; P<0.001; Fig. 4A). Gene interaction analyses through GenMANIA also showed that ALDH1L1 shared protein domains with *ALDH1A3*, that affected the TP53 pathway (Fig. 4B). Stratified analyses for the DFS showed that high *ALDH1L1* expression significantly decreased the risk of recurrence among patients >60 years of age, with a single tumor, BCLC stage 0/A, and AFP >300 ng/ml (Fig. 5A). Regarding the OS, high *ALDH1L1* expression significantly decreased the risk of death among patients in both male age groups, both tumor size groups with a single tumor, who

Table III. Association between risk factors and rs2276724 in HBV-related HCC patients.

Variables	TT	CT+CC	OR (95% CI)	P-value
Tumor size (cm)				
≤5	98	70	1	
>5	147	100	0.952 (0.640-1.418)	0.810
Tumor number				
Single	176	126	1	
Multiple	69	44	0.891 (0.573-1.386)	0.608
Child-Pugh score				
A	211	145	1	
B	34	25	1.070 (0.612-1.869)	0.812
BCLC stage				
A	136	100	1	
B	43	25	0.791 (0.453-1.379)	0.408
C	66	45	0.927 (0.586-1.467)	0.747
Radical resection[a]				
Yes	133	98	1	
None	103	69	0.909 (0.609-1.358)	0.642
AFP level[b]				
<400	122	88	1	
≥400	109	66	0.839 (0.557-1.266)	0.403
Regional invasion				
Absence	209	144	1	
Presence	36	26	1.048 (0.606-1.812)	0.866
PVTT				
No	199	143	1	
Yes	46	27	0.817 (0.485-1.376)	0.447
Pathological diagnosis[c]				
Well differentiated	13	11	1	
Moderately differentiated	198	143	0.854 (0.372-1.960)	0.709
Poorly differentiated	7	4	0.675 (0.156-2.930)	0.600

[a]Information regarding radical resection was unavailable for 12 patients; [b]information regarding pathological diagnosis was unavailable for 39 patients; [c]information regarding AFP level was unavailable for 30 patients. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; BCLC stage, Barcelona Clinic Liver Cancer stage; PVTT, portal vein tumor thrombus; AFP, α-fetoprotein;

were characterized with cirrhosis, BCLC stage 0/A, and AFP >300 ng/ml (Fig. 5B). We further analyzed the effects of *ALDH1L1* expression on the DFS and OS of GEO14520 HBV-related HCC patients by adjusting for age, sex, cirrhosis, BCLC stage and serum AFP level. The results showed that high *ALDH1L1* expression was significantly associated with a favorable prognosis for both the DFS and OS (adjusted P=0.04; adjusted HR=0.669; 95% CI=0.456-0.981 for DFS; adjusted P=0.001; adjusted HR=0.446; 95% CI=0.277-0.719 for OS; Table VI and Fig. 6A and B).

Joint-effect analyses among different *ALDH1L1* and *TP53* expression groups showed that patients with a high *ALDH1L1* and low *TP53* expression were significantly associated with a favorable prognosis, when compared with patients with a low *ALDH1L1* and *TP53* expression for both the DFS and OS (adjusted P=0.005; adjusted HR=0.460; 95% CI=0.266-0.795 for DFS; adjusted P=0.000011; adjusted HR=0.211; 95% CI=0.105-0.422 for OS; Table VII and Fig. 6C and D). Patients in groups b and d also had a reduced risk of death,

compared with patients with low *ALDH1L1* expression in the low TP53 expression group (adjusted P=0.023; adjusted HR=0.524; 95% CI=0.300-0.914 for group b; adjusted P=0.014; adjusted HR=0.434; 95% CI=0.222-0.846 for group d; Table VII and Fig. 6D).

Discussion

ALDH1L1 has been widely accepted as an astroglial marker in the brain (27,28) and is also expressed in neural stem cells (29), but is not a suitable marker for enteric glial cells (30). Due to the cell-specificity of ALDH1L1 in nerve cells, *ALDH1L1* polymorphisms have also been associated with neurological diseases, such as neural tube defects (31) and ischemic stroke (32), but *ALDH1L1* polymorphisms have not been investigated in spina bifida (33). It's upregulation is involved in central nervous system development and reduced proliferation (34). ALDH1L1 is mainly expressed in human liver (35), implying that ALDH1L1 has an important function
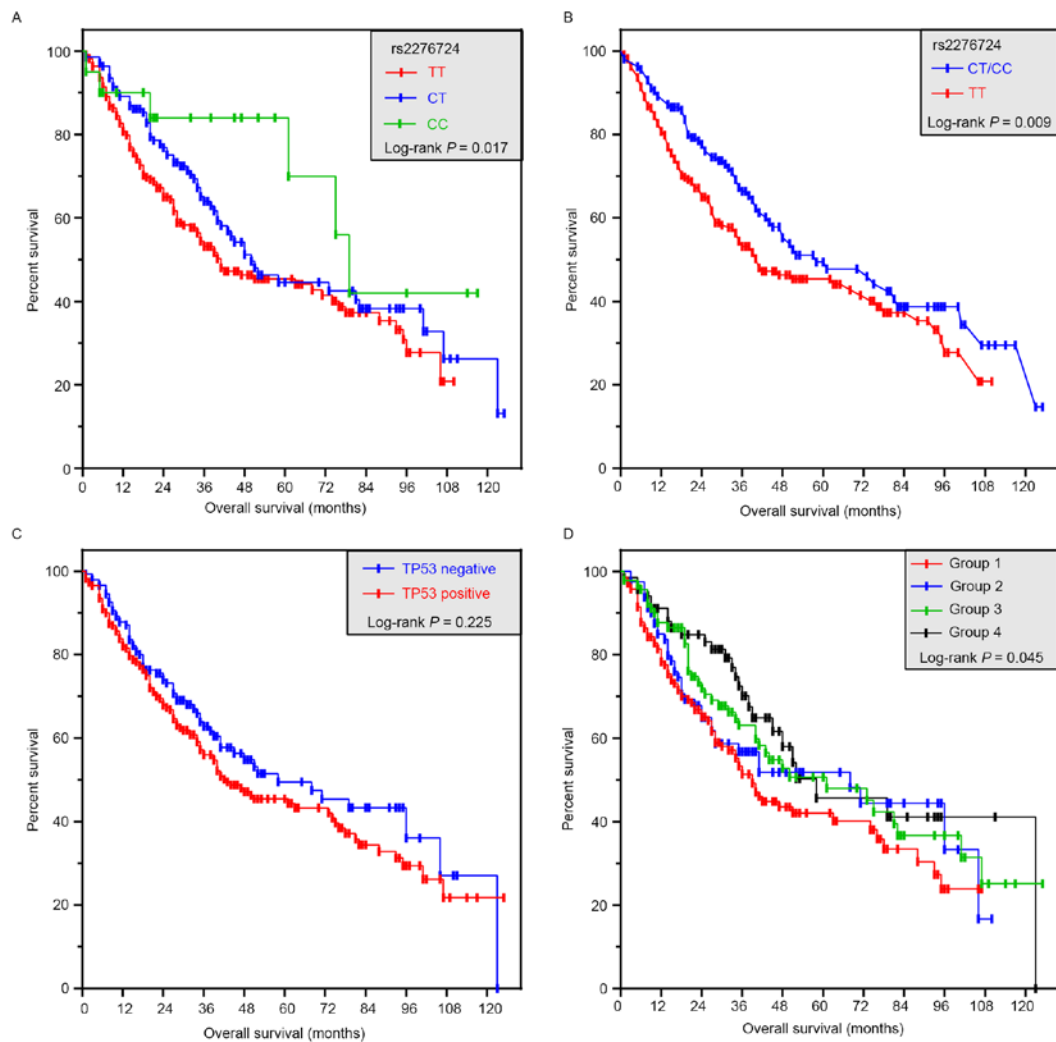
Figure 3. Survival curves of patients with rs2276724 and joint-effect analyses with different levels of TP53 expression. (A) Kaplan-Meier survival curves for patients with TT, CT, and CC genotypes. (B) Kaplan-Meier survival curves for patients with TT and CT/CC genotypes. (C) Kaplan-Meier survival curves for patients with different levels of *TP53* expression. (D) Kaplan-Meier survival curves for joint-effect analyses of patients with different rs2276724 genotypes and different levels of *TP53* expression. TP53, tumor protein p53.

Table IV. Survival analysis of HBV-related HCC patients according to rs2276724 and TP53 status.

Variable	Patients (n=415)	No. of events (%)	MST (months)	Crude HR (95% CI)	Crude p-value	Adjusted HR (95% CI)	Adjusted p-value[a]
rs2276724							
Allele							
T	640	309 (48.3)	41	1		1	
C	190	75 (39.5)	73	0.692 (0.535-0.893)	0.005	0.747 (0.572-0.976)	0.032
Genotype							
TT	245	123 (50.2)	39	1		1	
CT	150	63 (42.0)	50	0.716 (0.527-0.972)	0.032	0.749 (0.545-1.029)	0.074
CC	20	6 (30.0)	79	0.421 (0.185-0.958)	0.039	0.554 (0.240-1.278)	0.166
CT+CC	170	69 (40.6)	58	0.675 (0.502-0.909)	0.010	0.725 (0.533-0.986)	0.040
TP53 status							
Negative	162	68 (42.0)	58	1		1	
Positive	253	124 (49.0)	41	1.199 (0.892-1.612)	0.229	1.183 (0.872-1.605)	0.280

[a]Adjustment for drinking status, Child-Pugh score, tumor size, tumor number, BCLC stage, radical resection, cirrhosis, adjuvant antiviral treatment, PVTT in Cox proportion haphazard regression model. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TP53, tumor protein p53; MST, median survival time; HR, hazard ratio; CI, confidence interval.

Table V. Joint effect survival analysis of rs2276724 and different *TP53* expression statuses in HBV-related HCC patients.

Group	Genotype	TP53 status	Patients (n=421)	No. of events (%)	MST (months)	Crude HR (95% CI)	Crude p-value	Adjusted HR (95% CI)	Adjusted P-value[a]
1	TT	Positive	156	81 (51.9)	36	1		1	
2	TT	Negative	89	42 (47.2)	41	0.868 (0.598-1.260)	0.457	0.903 (0.610-1.336)	0.610
3	CT+CC	Positive	97	43 (44.3)	61	0.695 (0.479-1.008)	0.055	0.763 (0.518-1.126)	0.173
4	CT+CC	Negative	73	26 (35.6)	58	0.570 (0.366-0.888)	0.013	0.621 (0.396-0.973)	0.037

[a]Adjustment for drinking status, Child-Pugh score, tumor size, tumor number, BCLC stage, radical resection, cirrhosis, adjuvant antiviral treatment, PVTT in Cox proportion hazard regression model. HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MST, median survival time; HR, hazard ratio; CI, confidence interval.
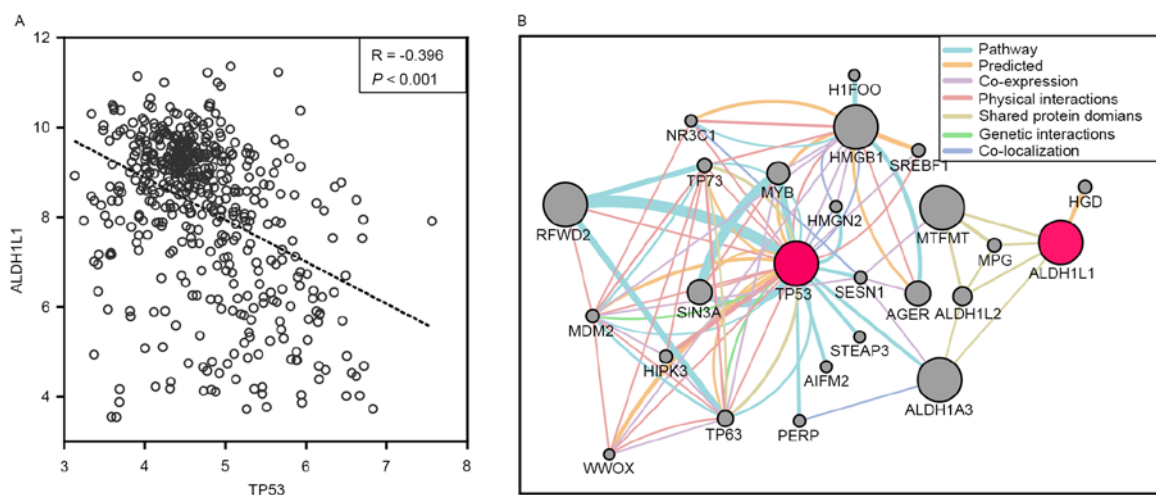

Figure 4. (A) Correlations between *ALDH1L1* and *TP53* mRNA expression using GSE14520. (B) A gene interaction diagram of the *ALDH1L1* and *TP53* genes using GeneMANIA. ALDH1L1, aldehyde dehydrogenase 1 family member L1; TP53, tumor protein p53.

in this organ. Consistent with our bioinformatic analyses, *ALDH1L1* is significantly downregulated in various human malignant tumors and cancer cell lines, including HCC (36). A similar result for *ALDH1L1* downregulation in different tumor tissues was confirmed by ONCOMINE analyses (37) and other studies (21,38,39), but in non-small cell lung cancer (NSCLC), *ALDH1L1* expression was upregulated (40). Our bioinformatic analyses also showed that *ALDH1L1* was downregulated in HBV-related HCC tumor tissues. *ALDH1L1* is upregulated in the presence of high concentrations of folate, and depletion of folate leads to the absence of *ALDH1L1*, resulting in cofilin dephosphorylation and inhibition of motility by protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) in several cell lines. These results suggested that folate promotes a malignant phenotype in cancer (41). However, a study of oral cancer reported that folate supplementation decreased the risk of oral cancer even with alcohol abuse, thus, ALDH1L1 may play a causal role in oral cancer occurrence (42). In spite of the conflicting results of these studies, underexpressed *ALDH1L1* was associated with an aggressive histology and/ or biological behavior in renal cell carcinomas and pilocytic astrocytomas (43,44). *ALDH1L1* knockdown in lung cancer cell lines showed that inhibition of *ALDH1L1* expression reduced adenosine triphosphate (ATP) production by

decreasing nicotinamide adenine dinucleotide (NADH) levels, resulting in cell death (40). Recent studies also reported that high expression of *ALDH1L1* is correlated with better survival in HCC (21), neuroblastoma (45) and breast cancer (BC) (46). However, survival analyses of gastric cancer showed the opposite result that high expression of *ALDH1L1* was associated with a worse prognosis (37,47). Furthermore, no significant relationship was observed between *ALDH1L1* mRNA expression and OS in NSCLC (48). As previously mentioned, *ALDH1L1* may play a different role as a tumor-suppressor during oncogenesis. Genetic variation analyses have reported that rs2276731 and rs2002287 of *ALDH1L1* can affect the risk of BC morbidity (n=1007) (49), but this conclusion was not found for the risk of prostate cancer (n=2288) including other ALDH1L1 SNPs (50). A study of HCC and lung cancer reported that *ALDH1L1* mRNA and protein levels correlated with the methylation status of the CpG island, and modicum ALDH1L1 CpG island methylation was sufficient to significantly decrease *ALDH1L1* expression, suggesting that the mechanism of action of *ALDH1L1* involves downregulation in cancers (51). A follow-up study in Chinese Kazakh patients with esophageal squamous cell carcinoma also showed that *ALDH1L1* is involved in a one carbon metabolic process that plays a key role in DNA methylation (39). A study carried out by
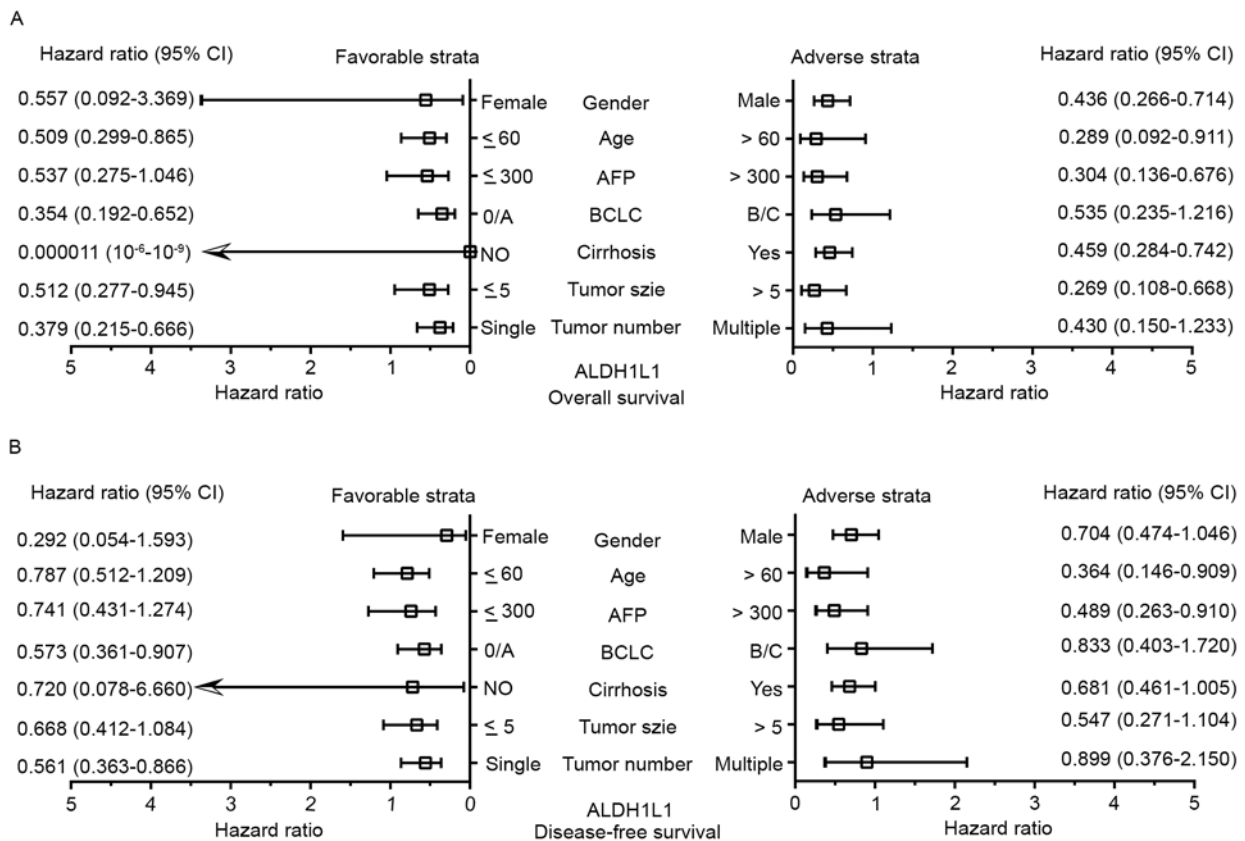
Figure 5. Stratified analyses of the associations of different *ALDH1L1* mRNA expression levels with the GSE14520 prognosis of HCC patients. All variables were stratified by favorable and adverse strata. (A) Stratified analysis between *ALDH1L1* and DFS. (B) Stratified analysis between *ALDH1L1* and the OS. ALDH1L1, aldehyde dehydrogenase 1 family member L1; HCC, hepatocellular carcinoma; DFS, disease-free survival; OS, overall survival.

Table VI. Survival analysis between *ALDH1L1* and *TP53* mRNA expression in GSE14520 HBV-related HCC patients.

Gene expression	Patients (n=218)	OS				DFS			
		No. of events (%)	MST (months)	Adjusted HR (95% CI)	Adjusted P-value[a]	No. of events	MST (months)	Adjusted HR (95% CI)	Adjusted P-value[a]
ALDH1L1									
Low	109	66 (60.6)	28	1		54 (49.5)	51	1	
High	109	55 (50.5)	53	0.669 (0.456-0.981)	0.040	30 (27.5)	NA	0.446 (0.277-0.719)	0.001
TP53									
Low	109	58 (53.2)	50	1		37 (33.9)	NA	1	
High	109	63 (57.8)	35	1.054 (0.726-1.5229)	0.783	47 (43.1)	NA	1.137 (0.722-1.791)	0.580

[a]Adjustment for age, sex, cirrhosis, BCLC stage, serum AFP level. ALDH1L1, aldehyde dehydrogenase 1 family member L1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; DFS, disease-free survival; OS, overall survival; MST, median survival time; HR, hazard ratio; CI, confidence interval.

Oleinik and Krupenko also reported that inducible *ALDH1L1* expression in A549 cells induced G1 cell cycle arrest and apoptosis. These anti-proliferative and apoptotic effects result in activation of *TP53*, followed by the TP53-mediated transcriptional activation of a downstream target of p21 (19), to function as a potent cyclin-dependent kinase inhibitor. Further studies of the relationship between *ALDH1L1* and *TP53* showed that expression of *ALDH1L1* induced suppressor effects that were

p53-dependent, and a TP53 deficit resulted in suppressor effects (20). This ALDH1L1-induced p53-dependent apoptosis also responded to folate stress, resulting in upregulation of ceramide synthesis (52).

A previous study of Guangxi HCC patients reported that *ALDH1L1* expression was associated with the prognosis of HCC (21). HBV-related HCC patients in Guangxi were associated with a high morbidity of HBV-infection (53).
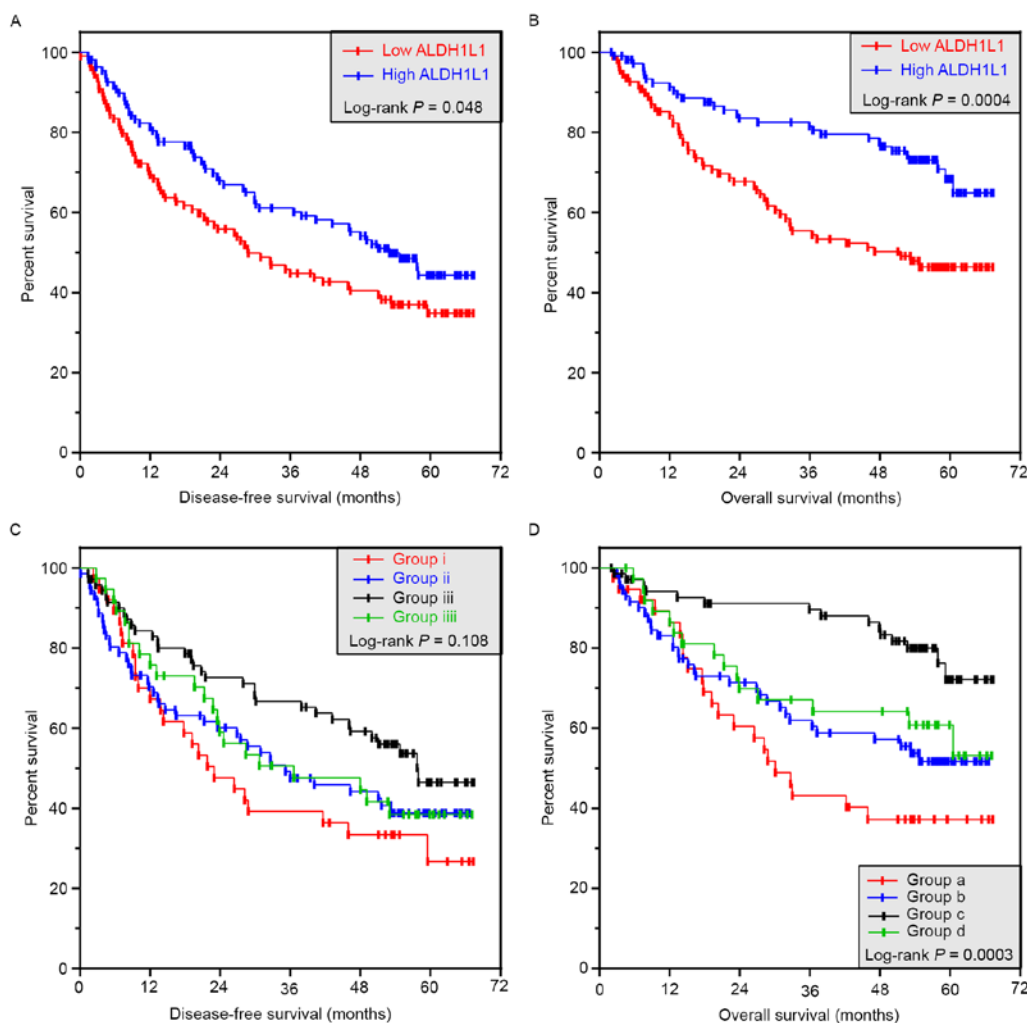
Figure 6. Survival curves for the GSE14520 analyses of HCC patients with different *ALDH1L1* mRNA expression levels, and the joint-effect analyses with *TP53* mRNA expression levels. (A) Kaplan-Meier survival curves for DFS for different *ALDH1L1* expression levels. (B) Kaplan-Meier survival curves for the OS analyses of different *ALDH1L1* expression levels. (C) Kaplan-Meier survival curves for the joint-effect analyses for different *ALDH1L1* and *TP53* mRNA expression levels; analysis for DFS. (D) Kaplan-Meier survival curves for the joint-effect analyses for different *ALDH1L1* and *TP53* mRNA expression levels; analysis for OS. HCC, hepatocellular carcinoma; TP53, tumor protein p53; ALDH1L1, aldehyde dehydrogenase 1 family member L1; DFS, disease-free survival; OS, overall survival.

Table VII. Joint effect survival analysis between ALDH1L1 and TP53 mRNA expression level in GSE14520 HBV-related HCC patients.

Group	ALDH1L1 expression	TP53 expression	Patients (n=218)	No. of events (%)	MST (months)	Adjusted HR (95% CI)	Adjusted P-value[a]
DFS							
i	Low	Low	38	25 (65.8)	23	1	
ii	Low	High	71	41 (57.7)	35	0.675 (0.406-1.122)	0.129
iii	High	Low	71	33 (46.5)	57	0.460 (0.266-0.795)	0.005
iiii	High	High	38	22 (57.9)	36	0.614 (0.342-1.101)	0.102
OS							
a	Low	Low	38	22 (57.9)	30	1	
b	Low	High	71	32 (45.1)	NA	0.524 (0.300-0.914)	0.023
c	High	Low	71	15 (21.1)	NA	0.211 (0.105-0.422)	0.000011
d	High	High	38	15 (39.5)	NA	0.434 (0.222-0.846)	0.014

[a]Adjustment for age, sex, cirrhosis, BCLC stage, serum AFP level. ALDH1L1, aldehyde dehydrogenase 1 family member L1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TP53, tumor protein p53; DFS, disease-free survival; OS, overall survival; MST, median survival time; HR, hazard ratio; CI, confidence interval.

The present study characterized *ALDH1L1* polymorphism in HBV-related HCC patients and its association with *TP53* expression. Bioinformatic analyses showed that rs2276724 S481G in *ALDH1L1* affected gene expression and was possibly deleterious to patients. We further analyzed the distribution of rs2276724 genotypes in different *TP53* expression groups and its possible association with the prognosis of HBV-related HCC patients. The results suggested that the occurrence of rs2276724 was similar between different *TP53* expression groups when analyzed in different genetic models. Survival analyses showed that the C allele was associated with a decreased risk of death in HBV-related HCC patients, when compared to the T allele. Through stratified analyses, the C allele carriers of rs2276724 had significantly decreased risk of death among patients with a tumor size ≤5 cm, a Child-Pugh score, and without a PVTT, BCLC stage B/C, non-radical resection, and the presence of regional invasion. Joint-effect analyses showed that the CT/CC of rs2276724 in TP53-negative patients was associated with a significantly decreased risk of mortality, compared to the TT of rs2276724 with TP53-positive patients. We then used the Chinese HBV-HCC mRNA expression profiling chip from the GSE14520 dataset to evaluate the prognosis of *ALDH1L1* expression in Chinese HBV-related HCC patients, and found that low *ALDH1L1* expression predicted a poor prognosis for Chinese HBV-related HCC patients with low expression of HBV-related HCC tumor tissues. High *ALDH1L1* expression significantly decreased the risk of HCC recurrence among patients with an age >60 years, a single tumor, BCLC stage 0/A, AFP >300 ng/ml, and showed a decreased risk of mortality among the male HCC patients in both age groups, both tumor size groups, a single tumor with cirrhosis, with BCLC stage 0/A, and an AFP >300 ng/ml. Gene interaction analyses showed that GeneMANIA *ALDH1L1* and *TP53* expression mRNA levels were negatively correlated in Chinese HBV-related HCC patients, and further showed that *ALDH1L1* shared a protein domain with *ALDH1A3* that was involved in the TP53 pathway. We combined the analyses of *ALDH1L1* and *TP53* expression in HBV-related HCC patients to show that high *ALDH1L1* with low *TP53* expression was associated with a significantly decreased risk of HBV-related HCC recurrence and mortality when compared with low *ALDH1L1* and low *TP53* expression. Patients with high *TP53* expression also had a significantly decreased risk of HBV-related HCC death, compared with low *ALDH1L1* and low *TP53*-expressing patients.

In conclusion, the present study showed, for the first time, that prognosis can be predicted for the rs2276724 genotypes of *ALDH1L1* in HBV-related HCC patients and their associations with *TP53* expression. The CT/TT genotype of rs2276724 may have a protective survival value and may be a potential prognostic marker in patients with HBV-related HCC receiving hepatic resection. We also confirmed that a decrease in *ALDH1L1* expression predicts a poor prognosis for patients with HBV-related HCC. The expression of *ALDH1L1* and genotypes of rs2276724 may therefore play a role in *TP53* expression in HBV-related HCC of Chinese hepatic resection patients. Due to the limitations of the relatively small sample sizes and the long period of specimen collection, we did not analyze the association among rs2276724 genotypes and

mRNA expression. Further well-designed, comprehensive, and large sample size studies are therefore needed to confirm our results.

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