Effects of Gene Polymorphisms, Metabolic Activity, and Content of Alcohol Dehydrogenase and Acetaldehyde Dehydrogenases on Prognosis of Hepatocellular Carcinoma Patients

Na Gao, Jingjing Chen, Bing Qi, Tianyuan Zhao, Yuanyuan Guo, Yan Fang, Zixinying Han, Hai-ling Qiao

Institute of Clinical Pharmacology, Zhengzhou University, Zhengzhou, Henan, China

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ABSTRACT

Background: *Alcohol dehydrogenase and acetaldehyde dehydrogenases have been associated with hepatocellular carcinoma, but how alcohol dehydrogenase and acetaldehyde dehydrogenases alter the prognosis of hepatocellular carcinoma have not been completely elucidated.*

Methods: *Metabolic activities, gene polymorphisms, and content of alcohol dehydrogenase and acetaldehyde dehydrogenases were determined in 68 fibrotic livers from hepatocellular carcinoma patients. These characteristics were then correlated with clinical features and prognosis in these patients.*

Results: *The median survival time of the ALDH-high activity group (727 days) was increased by 128% compared with that of ALDH-low activity group (319 days), and there was a significant negative correlation between the activity of acetaldehyde dehydrogenases and the level of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. There was no difference in survival time between ALDH2-high and ALDH2-low expression group, though the activity of acetaldehyde dehydrogenases had correlation with the content of ALDH2 ($r = 0.6887$, $P < .001$). Mutation at ALDH2rs671 significantly decreased both the activity and content of acetaldehyde dehydrogenases, but the polymorphism had no relationship with progression of hepatocellular carcinoma patients. In addition, the activity and 3 polymorphisms of alcohol dehydrogenase had no effect on overall survival. Mutation at ADH1Crs698 significantly decreased both the activity and content of alcohol dehydrogenase ($P < .05$), mutation at ADH1C rs2241894 had an inverse effect, and mutation at ADH1B rs1229984 increased activity but did not affect content. The activity of alcohol dehydrogenase had a moderate correlation with the content of ADH1A and ADH1C in livers ($P < .05$).*

Conclusion: *Low activity of acetaldehyde dehydrogenases in livers correlates with poor prognosis and clinical progression in hepatocellular carcinoma patients, and both gene polymorphisms and content influence its metabolic activity.*

Keywords: *ADH, ALDH, hepatocellular carcinoma, polymorphism, prognosis*

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death worldwide in 2018.[1] In patients who underwent radical resection, the 5-year survival rate has remained below 12% due to postoperative recurrence and/or metastasis.[2,3] It is vital to identify the factors that affect recurrence and survival of HCC patients. Recently, metabolic alterations have been shown to play a major role in tumorigenesis.[4-7] Therefore, changes in enzymes involved in metabolism are receiving increasing attention.

Alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenases (ALDH) are classified as phase I xenobiotic metabolizing enzymes, and they take part in the metabolism of ethanol, retinol, hydroxysteroids, lipid aldehyde, and so on.[8,9] Currently, a considerable amount of studies have focused on the effects of ADH and ALDH on liver disease. The results of Jelski et al[10] showed that total ADH and ALDH activities were significantly higher in cancer tissues than in healthy tissues from HCC patients. However, Hou et al[4] found that ALDH2 levels were significantly lower in liver tumor tissues. Yi et al[11] indicated that ADHIII can promote liver fibrosis by activation of hepatic stellate cells and inhibition of natural killer cell activity. Our previous results showed that the activities of total ADH, ADHI, and ADHII in fibrotic livers increased significantly, and both the positive rate of ADHI and ADHII were more than 70%[6]. Guo et al[12] revealed that ALDH2 can ameliorate liver injury caused by chronic alcohol intake in ALDH2 transgenic mice.

Corresponding author: ***Hai-ling Qiao****, e-mail:* ***qiaohl@zzu.edu.cn***

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It is well known that there are clear inter-individual variations in the activities of ADH and ALDH.[6,10] Gene polymorphisms are considered to have important effect on enzyme activity.[13,14] The genes encoding *ADH1B*, *ADH1C*, and *ALDH2* exhibit polymorphisms with ethnic variations. Single-nucleotide polymorphisms of *ADH1B rs1229984* (*ADH1B*2*), *ADH1Crs698(A>G)*, and *ALDH2* rs671 (*ALDH2*2*) receive more attention.[15] Many studies have demonstrated that these polymorphisms are related to some diseases, including esophageal cancer,[16] hypopharyngeal squamous cell carcinoma,[17] and pancreatitis.[18] In addition, protein content is another important factor affecting enzyme activity. Prasad et al[19] determined the expression levels of ADH and ALDH in alcoholic and hepatitis C cirrhotic livers, and considerable individual variation was found in ADH protein content.

The above data indicate that ADH and ALDH likely play important roles in liver diseases, especially HCC. Moreover, gene polymorphisms and expression might affect their activity. However, their specific function during HCC development remains poorly understood. To our knowledge, there are only 2 reports that focused on the relationship between ALDH and progression of HCC.[4,20] The results of Hou et al[4] suggested that decreased levels of ALDH2 in tumor tissues may exhibit a poor prognosis in HCC patients, and forcing the expression of ALDH2 in HCC cells inhibited their aggressive behavior. Huang et al[20] reported that HCC patients carrying mutant allele (*rs*671 G>A), a defective allele, of *ALDH2* had a favorable postoperative outcome. Their results seem to be contradictory because the expression level of ALDH2 in GA and AA genotype carriers is only between 30% and 40% of that of wild type.[21]

In this study, gene polymorphisms, protein content, and the activities of ADH and ALDH were analyzed in 68 human fibrotic liver samples from HCC patients, and the relationship between content and activity was assessed. Moreover, the effects of ADH and ALDH on overall survival (OS) were analyzed.

Main Points

- *Both gene polymorphisms and content influence the activity of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenases (ALDH).*
- *Polymorphisms of ADH and ALDH have no effects on overall survival of hepatocellular carcinoma (HCC) patients.*
- *Low activity of ALDH in livers correlates with poor prognosis in HCC patients.*

MATERIALS AND METHODS

Patients and Follow-up

The research has been approved by the ethics committee. Human liver tissues from 68 patients with HCC receiving surgical resection were collected after written informed consent (Table 1) from 2013 to 2014.[7,22,23] All liver samples were from patients with severe fibrosis (S3 or S4) as confirmed by the pathological results.[22] The liver samples were 2 cm distant from the tumor tissue and were placed in liquid nitrogen within 30 minutes of resection. Clinical characteristics of the patients were recorded, including gender, age, alpha-fetoprotein (AFP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), HBV surface antigen, tumor size, and tumor volume. All cases were followed up after surgical treatment. The OS was calculated based on the date of death or the last visit time (December 31, 2017).

Table 1. Clinical and Laboratory Characteristics of Hepatocellular Carcinoma Patients

Variables	Group	Number	Percentage
Gender	Male	58	85.29
	Female	10	14.71
Age	Age < 40 years	8	11.76
	40 ≤ Age ≤ 60 years	42	61.76
	Age >60 years	18	26.47
Smoking	Non-smoker	38	55.89
	Smoker	30	44.11
Alcohol	Non-drinker	46	67.65
	Drinker	22	32.35
ALT	<40.00	39	57.35
	>40.00	29	42.65
AST	<40.00	31	45.59
	>40.00	37	54.41
ALP	30.00-120.00	44	64.71
	>120.00	24	35.29
S3/S4	S3	23	33.82
	S4	45	66.18
Total		68	100

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

Determination of Total ADH and ALDH Activity in Livers[6,10,24,25]

The activities of total ADH and ALDH were measured according to the method reported previously. In brief, the substrates were *p*-nitrosodimethylaniline, and 6-methoxy-2-naphthaldehyde, respectively. Wavelength of 440 nm was used for assays of total ADH. Emission wavelength of 360 nm with excitation wavelength of 310 nm was used for assays of total ALDH.

Determination of the Content of ADH and ALDH2 in Livers

Label-free quantitative proteomics method was used for quantifying liver tissue proteins.[26] The lysate of the protease mixed inhibitor was added to liver tissue and placed in an ice bath for 20 minutes after brief sonication. The preparation was subjected to centrifugation at 4° and 16 000 g × 10 minutes to obtain the supernatant. The protein concentration was measured using a BCA kit (Boster, Wuhan, China). Trypsin was added for digestion, and the Nanodrop micro-ultraviolet spectrophotometer (Thermo, Madison, WI, USA) was used to detect the peptide concentration. Peptide fractions were pre-separated using reverse chromatography columns and sequenced on a Q-Exactive HF mass spectrometer (Thermo). A peptide solution produced from HEK293T cells was used as quality control. The data analysis was performed using MaxQuant software (version 1.5.3.8), and the normalized intensity-based absolute quantification (iBAQ) value based on peak intensity represented the protein expression.

Determination of Polymorphisms of ADH and ALDH

Polymorphisms *ADH1B rs1229984* (*G>A*) and *ADH1Crs698 (A>G)* were determined by polymerase chain reaction-restriction fragment length polymorphism.[27] *ADH1C rs2241894 (A>G)*, *ALDH2 rs671 (A>G)*, and *ALDH2 rs13306164 (C>T)* were detected by performing mass spectrometry (LIUHE HUADA Genomics Technology Co., Ltd., Beijing, China).

Statistical Analysis

Statistical analyses were performed by using the Statistical Package for Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA), and a value of $P < .05$ was considered as a significant difference. Alcohol dehydrogenase and ALDH activities were analyzed by Mann–Whitney *U* test due to abnormal distribution. Enzyme content was analyzed with independent-samples *t* tests between 2 groups or one-way ANOVA among more than 2 groups. Survival analysis was performed using the Kaplan–Meier method, and the difference between 2 groups was compared by the log-rank test.

RESULTS

The Activity of ADH and ALDH in HCC Patients

The metabolic activities of total ADH and ALDH were measured in 68 liver homogenates. Generally, the metabolic activities of ADH and ALDH are expressed by per mg of homogenate protein (V_P), which ignore the difference in the content of homogenate protein in 1 g of liver tissues (HPGL) in different samples. So in this study, the metabolic activities were based on per gram of liver (V_L), and V_L was calculated by multiplying V_P and the values of HPGL (Figure 1A). The minimal and maximal values of HPGL were 45.1 and 107.6 mg protein/g liver, respectively, representing a 2.4-fold variation. There were also substantial individual variations in metabolic activities of total ADH and ALDH. The individual variations in V_L of ADH reached 36-fold, while the variations in V_L at 95% prediction interval decreased to less than 23-fold. Compared with ADH, the fold change of ALDH V_L was much lower but still achieved 10-fold.

The correlations between the activities of ADH and ALDH were analyzed (Figure 1B), and the results showed that the activity of ADH had no significant correlation with ALDH ($P > .05$).

Effects of Gene Polymorphisms on Activity of ADH and ALDH

Alcohol dehydrogenase I comprises 3 subtypes, including ADH1A, ADH1B, and ADH1C. Three mutations were detected and the effects on the corresponding enzyme activities were shown in Table 2. The results displayed that the mutation frequency of *ADH1B rs1229984*, *ADH1Crs698*, and *ADH1C rs2241894* correspondingly were 62.0%, 9.7%, and 71.6%, respectively. The *ADH1B rs1229984* mutation increased ADH activity compared to the wild-type *GG* genotype, and the *ADH1C rs698* mutation reduced ADH activity ($P < .05$). The V_L of individuals with mutant homozygous and heterozygous alleles for the *ADH1C rs2241894 (A > G)* was increased by 70.9% and 95% compared with the *AA* allele.

The *ALDH2 rs671* mutation frequency was 15%, and *ALDH2 rs13306164* mutation frequency was 5.2% (Table 2). The *ALDH2 rs671* mutation significantly decreased the activity of ALDH ($P < .01$). The V_L of ALDH decreased by 62% in the *GA* genotype compared with the

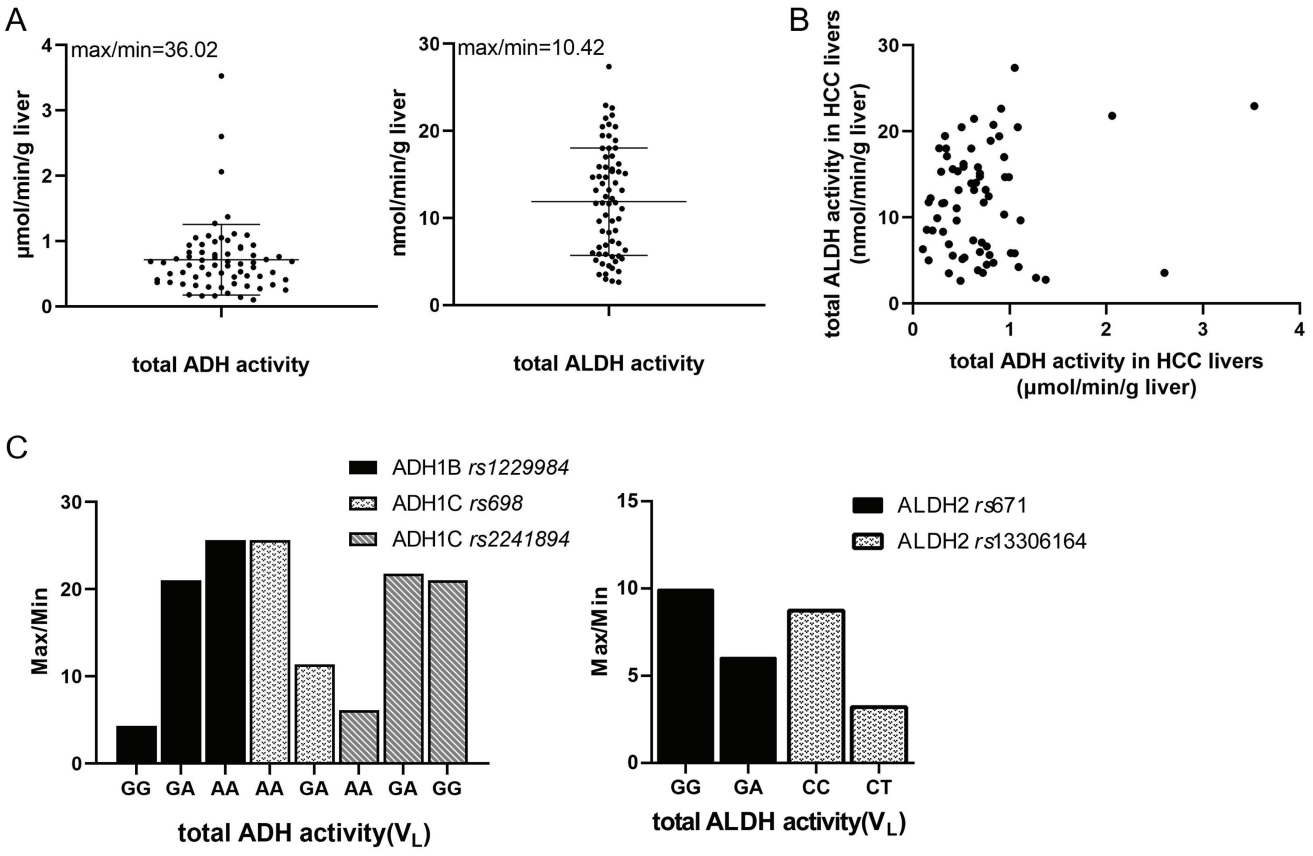

Figure 1. (A) The activity of ADH and ALDH. (B) The correlation between ADH and ALDH activity. (C) C Individual variations in subjects with the same genotype in ADH and ALDH. Max/min: ratio of maximum to minimum of V_L. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenases; HCC, hepatocellular carcinoma.

Table 2. The Distribution of Activity and Content of ADH of ALDH According to Polymorphism Types (Median)

					Content	
	Allele	n	Frequency (%)	V_L	n	iBAQ ($\times 10^8$)
ADH1B rs1229984	GG	10	14.9	485.76	6	55.5
	GA	31	46.3	733.61*	18	51.4
	AA	26	38.8	633.07	15	51.4
ADH1C rs698	AA	54	80.6	680.58	33	6.30
	GA	13	19.4	450.78*	6	3.60***
ADH1C rs2241894	AA	9	13.4	373.44	6	4.27
	GA	20	29.9	728.09*	14	6.30*
	GG	38	56.7	638.23*	19	5.93*
ALDH2 *rs671*	GG	47	70.1	14.69	25	20.8
	GA	20	29.9	5.61**	14	8.62***
ALDH2 *rs13306164*	CC	60	89.6	11.71	36	17.7
	TC	7	10.4	13.18	3	27.7

V_L, nmol/min/g tissue; *$P < .05$, **$P < .01$, ***$P < .001$ versus wild-type homozygotes.
ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenases; iBAQ, intensity-based absolute quantification.

GG genotype. The *ALDH2 rs13306164* mutation had no significant effect on the V_L of ALDH.

Though gene polymorphisms had an effect on activity, there were still substantial differences among the subjects with the same genotype (Figure 1C). For the V_L of ADH, individual variation in subjects with *ADH1Brs1229984 GG* genotype was lowest, and it was still 4.3-fold. Moreover, the variation in subjects with *ADH1Crs698 AA* genotype was greatest which was up to 26-fold. For the V_L of ALDH, the variation of the *CC* genotype based on *rs13306164* was 9-fold.

Effects of Content on Activity of ADH, ALDH, and ALDH2

The protein content of ADH1A, ADH1B, ADH1C, and ALDH2 of 39 samples was detected in 68 samples and expressed by iBAQ. The correlation between the expression level and the corresponding activity was evaluated, and the results showed that there was a moderate correlation between the activity of ADH and the expression level of ADH1A and ADH1C (Figure 2, $P < .05$). Compared with ADH, the correlations between ALDH2 content and ALDH activity were higher with a correlation coefficient of 0.6887 (Figure 2). Furthermore, there was a correlated trend between the ADH activity and content of ADH1B, and the *P* value was close to .05. The correlations between them were not statistically significant which might be due to the relatively small sample size.

As shown in Table 2, individuals who possessed *GA* alleles of *ADH1C rs698* had obviously lower ADH1C protein levels than those with the *AA* genotype ($P < .05$). The protein level of ADH1C in samples with the *G* allele was significantly higher than that in samples carrying the *AA* allele for *ADH1C rs2241894* ($P < .05$). For the ALDH2 polymorphism, the *ALDH2 rs671* mutation decreased the content by 58.6%. The change trend and effect on the content of the 3 polymorphisms were similar with the effect on corresponding activity.

Effects of ADH, ALDH, and ALDH2 on the Clinical Characteristics of HCC Patients

In order to analyze the effects of ADH and ALDH on the development and progression of HCC, the patients were divided into 2 groups based on the median activity: a high-activity group and a low-activity group. The difference in clinical characteristics, such as ALT, AST, ALP, AFP, tumor size, and tumor volume, between the groups was analyzed. The results showed that the patients with high ALDH activity achieved better OS and a longer time to death than those in the ALDH-low activity group (Figure 3A). The median survival time of the ALDH-low activity group and the ALDH-high activity group was 319 days and 727 days, respectively. Compared with the low-activity group, the survival time of the high-activity group was 128% longer. In addition, there was a significant correlation between the activity of ALDH and several indicators of liver injury, including ALT, AST, and ALP (Figure 3C–E, $P < .05$). The multivariate Cox regression analysis further indicated that ALDH activity was an independent risk factor for OS in patients with HCC (Table 3). The relationship between ALDH2 content and survival in patients was also analyzed. The median survival time of the ALDH-low expression group (n = 19) and the ALDH-high expression group (n = 20) was 351 days and 344 days, respectively ($P > .05$, Figure 3B).

The effects of gene polymorphisms in ADH and ALDH, including *ADH1B rs1229984*, *ADH1Crs698*, *ADH1C rs2241894*, *ALDH2 rs671*, and *ALDH2 rs13306164* on OS and other clinical characteristics were analyzed. The results showed that there were no significant differences among different genotypes ($P > .05$, Figure 4A and B).

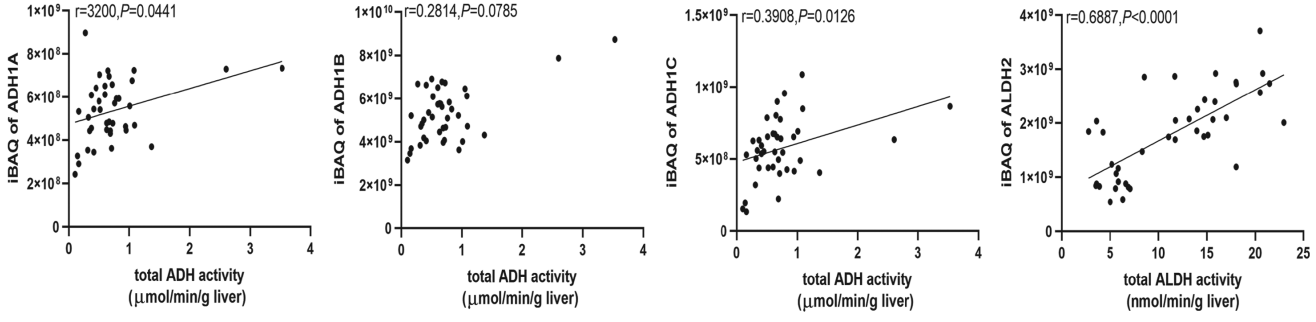

Figure 2. Correlation between content and activity of ADH and ALDH in different human livers (n = 39). The content was expressed as iBAQ value. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenases; iBAQ, intensity-based absolute quantification.

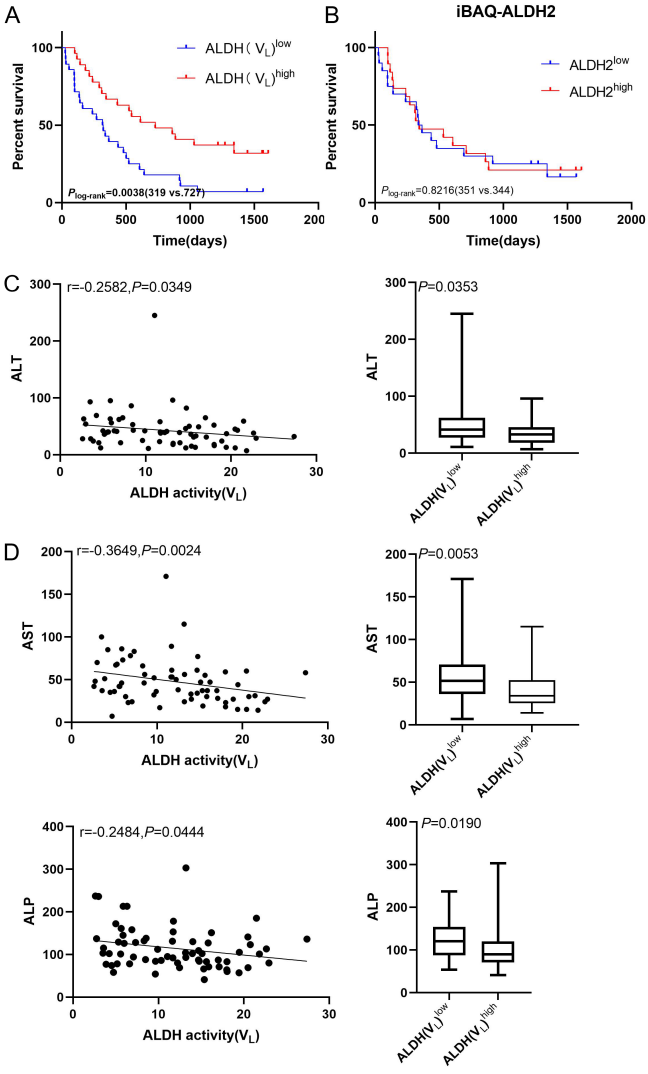

Figure 3. The survival curves of the HCC patients according to ALDH activity and levels. (A) Kaplan–Meier survival curves for ALDH activity (A) and content (B) in all patients with HCC. (C–E) Analysis of the correlation and differences between ALDH activity subgroups and clinical indicators ALT (C), AST (D), ALP (E). ALD, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenases; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HCC, hepatocellular carcinoma.

DISCUSSION

Hepatocellular carcinoma is the most common form of liver cancer characterized by a high recurrence rate and a poor prognosis. Most risk factors can lead to the formation of liver fibrosis and further development of fibrosis or cirrhosis, which is shown in between 80% and 90% of patients with HCC.[2] Thus, the status of fibrosis liver tissue surrounding the tumor might be very important for the recurrence and the clinical outcome of these patients after surgical resection. In this study, the activities of ADH and ALDH in 68 livers from HCC patients were determined based on liver tissues and showed substantial variation, reaching 36-fold. The influence of gene polymorphisms and content on inter-individual variations in metabolic activities was systematically investigated. We found that low ALDH activity in livers correlates with poor prognosis and clinical progression. These results are useful for the development of individualized treatment for patients with HCC.

As mentioned above, many papers have focused on the relationship between ADH, ALDH, and liver disease.[4,6,10-12] Yi et al[11] and Guo et al[12] focused on the effects of ADHIII and ALDH2 on liver fibrosis and alcohol-induced hepatic steatosis, respectively, and did not address HCC. Three other studies were conducted with HCC patients.[4,6,10] Several examined the enzymes in tumor tissue or in the adjacent tumor tissue, namely fibrosis liver. They measured either enzyme activity or content. We think that the activity of the metabolic enzymes in fibrotic tissues (adjacent tumor tissues) of HCC patients may be more meaningful for the prognosis of patients. The main reasons are as follows. First, HCC is different from other cancers because the adjacent tumor tissues of between 80% and 90% of HCC patients are fibrotic tissues rather than normal tissues.[2] This is the "soil" for tumor cell, first suggested in Paget's "seed and soil" hypothesis.[28] By studying the adjacent tumor liver tissue, risk factors that affect the development of HCC may be found. For example, we have carried out a series of studies on adjacent tumor tissues in HCC patients and have found that CYP2E1 activity can affect the occurrence and prognosis of HCC.[22,23] Secondly, compared with the whole liver, a tumor takes up a small proportion, so the adjacent tumor tissues can better reflect the overall condition of the metabolic enzyme in the liver. Lastly, enzyme activity is more representative of enzyme characteristics than enzyme content because the content is not the only factor that affects enzyme activity.

Our previous data indicated that the ADH activity was significantly increased in fibrotic livers from HCC patients compared with normal livers, and a higher level of ADH activity may be a risk factor for hepatofibrogenesis.[6] However, in this study, we failed to find an association between ADH activity and progression of HCC, which suggested that the occurrence and progression of HCC were not completely consistent.

Table 3. Univariate and Multivariate Analyses—Cox Regression Overall Survival

Clinical Parameters	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
ALDH activity*	0.949 (0.904-0.997)	.039	0.941 (0.889-0.996)	.036
Gender, male = 1	1.209 (0.538-2.715)	.646		.394
Age (years)	1.004 (0.979-1.030)	.760		.715
Smoking	0.662 (0.363-1.207)	.176		.586
Drinking	0.894 (0.472-1.693)	.732		.484
HBsAg	0.314 (0.076-1.308)	.093	0.457 (0.107-1.957)	.299
ALT	1.006 (1.000-1.013)	.052	0.974 (0.957-0.990)	.002
AST	1.016 (1.008-1.024)	<.001	1.052 (1.027-1.077)	<.001
ALP	1.003 (0.998-1.008)	.278		.466
GGT	1.002 (0.999-1.005)	.122		.546

*The activity of ALDH based on tissue level.
HR, hazard ratio; ALDH, acetaldehyde dehydrogenases; HBsAg, HBV surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptadase.

We found that patients with high ALDH activity achieved better OS. Mutation of rs671 significantly decreases ALDH activity, but there was still a substantial difference among the subjects with the same genotype. The results indicated that the polymorphism had a limited effect on activity. Furthermore, though ALDH activity had high correlation with content, the content was not the only factor that causes individual variations in activity. This result explained why ALDH activity was related to survival, while the influencing factors, the content and polymorphism,

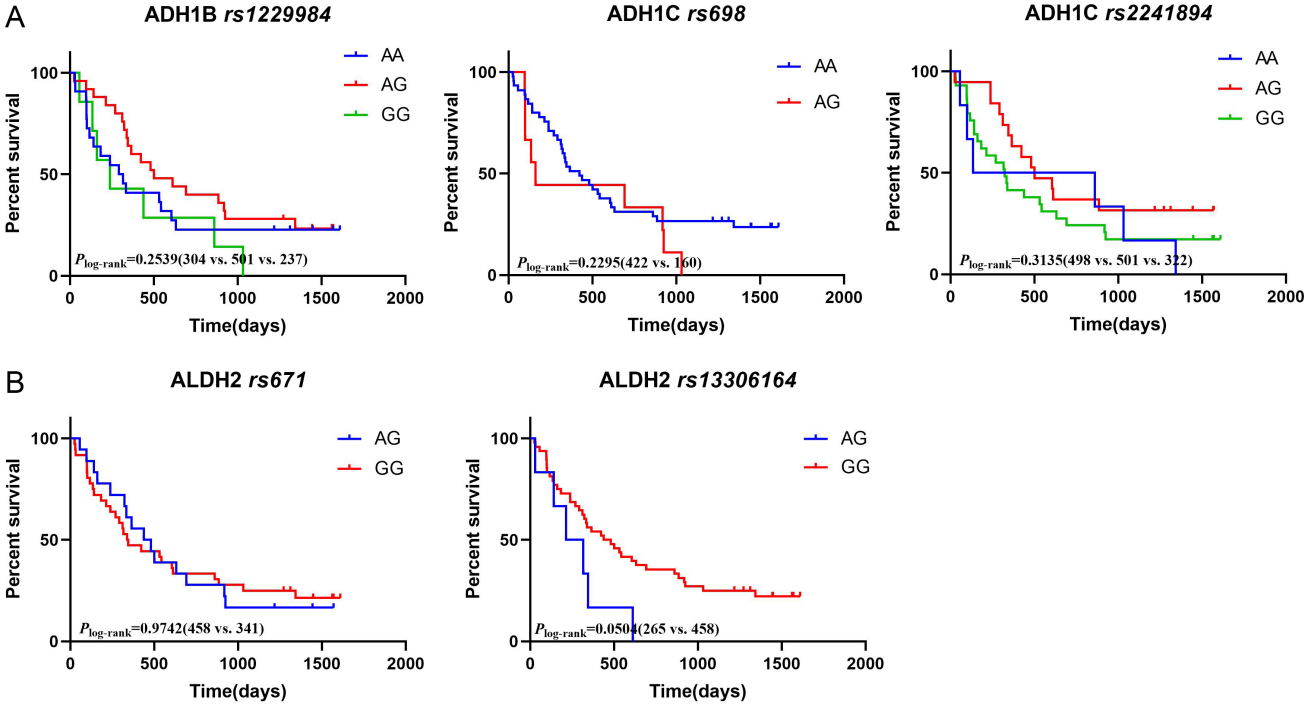

Figure 4. Kaplan–Meier survival curves for gene polymorphisms of ADH (A) and ALDH (B) in patients with HCC. ALD, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenases; HCC, hepatocellular carcinoma.

had no effect on survival to a certain extent. The results of Hou et al[4] showed that the decreased levels of ALDH2 might indicate a poor prognosis in HCC patients, which were not completely consistent with ours. The reason might be that Hou[4] measured the content of ALDH in tumor tissues, while we measure the content in adjacent tumor tissues. In addition, this result was different from the study of Huang et al[20] in which HCC patients carrying a defective allele of *ALDH2* had a favorable postoperative outcome. The differing results might be due to the sample size and the stage of cancer and therapies with curative intent.

Most of the studies about polymorphism of ADH and ALDH focus on alcohol metabolism or alcoholism. ADH1B*2 and ALDH2*2 have been reported to show protective effects against developing alcoholism.[29,30] Individuals with the ADH1B*2 allele showed a higher alcohol elimination rate.[31] Marshall et al[32] reported that ADH1B*3 had a marginal effect on alcohol pharmacokinetics in nondependent drinkers of African descent. Other reports have shown that polymorphisms in ALDH2 dramatically influence blood ethanol and acetaldehyde levels.[33,34] However, pharmacokinetics included absorption, distribution, metabolism, and excretion, and the in vivo studies could not really reflect the effects of gene polymorphisms on metabolism.[13] A few studies existed regarding the effects of gene polymorphisms on ADH and ALDH activities in vitro. Enomoto et al[35] analyzed the effect of ALDH2*2 on hepatic ALDH2 activity in 23 patients and found that hepatic ALDH2 activity was not evident in the 2 cases of the mutant homozygote, which indicated that ALDH2*2 causes a catalytic inactivation of the enzyme. Our study systematically evaluated the influence of gene polymorphism of ADH and ALDH on metabolic activity of ADH and ALDH in vitro, and we found that the mutation of ADH1C *rs698* and ALDH2 *rs671* significantly decreases the activity of corresponding enzyme, while ADH1B *rs1229984* had the opposite effect.

Moreover, the effects of ADH and ALDH mutations on content were also analyzed. Interestingly, we found that the polymorphism locus of *ADH1C rs698, ADH1C rs2241894, and ALDH2 rs671* significantly affected the content of the corresponding enzyme, and the extent of the effect on content was consistent with that on activity. This suggests that the effects of the above polymorphisms on activity were achieved by the effects on the content. While *ADH1B rs1229984* only increased the activity, not content, this locus might directly affect enzyme activity.

Despite these encouraging results, several limitations in our study should be stated. First, only HCC patients with serious fibrosis (S3 and S4) undergoing resection were enrolled. Second, the expression levels of ADH and ALDH were iBAQ values, which was not absolute content.

In conclusion, the study suggests that low activity of ALDH in livers correlates with poor prognosis and clinical progression in HCC patients, and both gene polymorphisms and content influence its metabolic activity.

Ethics Committee Approval: The study was approved by the Ethics Committee of Zhengzhou University.

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Concept – H.Q.; Design – H.Q., N.G.; Supervision – Y.G.; Resources – B.Q., T.Z.; Materials – Z.H., Y.F.; Data Collection – B.Q., Y.G.; Analysis – N.G., J.C., B.Q.; Writing Manuscript – N.G., J.C.; Critical Review – H.Q.

Declaration of Interest: The authors have no conflict of interest to declare.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. [CrossRef]

2. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118-1127. [CrossRef]

3. Zhou L, Rui JA, Zhou WX, Wang SB, Chen SG, Qu Q. Edmondson-Steiner grade: a crucial predictor of recurrence and survival in hepatocellular carcinoma without microvascular invasion. Pathol Res Pract. 2017;213(7):824-830. [CrossRef]

4. Hou G, Chen L, Liu G, et al. Aldehyde dehydrogenase-2 (ALDH2) opposes hepatocellular carcinoma progression by regulating AMP-activated protein kinase signaling in mice. Hepatology. 2017;65(5):1628-1644. [CrossRef]

5. Patterson AD, Maurhofer O, Beyoglu D, et al. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. Cancer Res. 2011;71(21):6590-6600. [CrossRef]

6. Gao N, Li J, Li MR, et al. Higher activity of alcohol dehydrogenase is correlated with hepatic fibrogenesis. J Pharmacol Exp Ther. 2018;367(3):473-482. [CrossRef]

7. Gao J, Wang Z, Wang GJ, et al. From hepatofibrosis to hepatocarcinogenesis: higher cytochrome P450 2E1 activity is a potential risk factor. Mol Carcinog. 2018;57(10):1371-1382. [CrossRef]

8. Sung CK, Kim SM, Oh CJ, Yang SA, Han BH, Mo EK. Taraxerone enhances alcohol oxidation via increases of alcohol dehyderogenase

(ADH) and acetaldehyde dehydrogenase (ALDH) activities and gene expressions. Food Chem Toxicol. 2012;50(7):2508-2514. [CrossRef]
9. Song BJ, Akbar M, Jo I, Hardwick JP, Abdelmegeed MA. Translational implications of the alcohol-metabolizing enzymes, including cytochrome P450-2E1, in alcoholic and nonalcoholic liver disease. Adv Pharmacol. 2015;74:303-372. [CrossRef]
10. Jelski W, Zalewski B, Szmitkowski M. The activity of class I, II, III, and IV alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in liver cancer. Dig Dis Sci. 2008;53(9):2550-2555. [CrossRef]
11. Yi HS, Lee YS, Byun JS, et al. Alcohol dehydrogenase III exacerbates liver fibrosis by enhancing stellate cell activation and suppressing natural killer cells in mice. Hepatology. 2014;60(3):1044-1053. [CrossRef]
12. Guo R, Xu X, Babcock SA, Zhang Y, Ren J. Aldehyde dedydrogenase-2 plays a beneficial role in ameliorating chronic alcohol-induced hepatic steatosis and inflammation through regulation of autophagy. J Hepatol. 2015;62(3):647-656. [CrossRef]
13. Gao N, Tian X, Fang Y, et al. Gene polymorphisms and contents of cytochrome P450s have only limited effects on metabolic activities in human liver microsomes. Eur J Pharm Sci. 2016;92:86-97. [CrossRef]
14. Adeniji EA, Olotu FA, Soliman MES. Alcohol metabolic inefficiency: structural characterization of polymorphism-induced ALDH2 dysfunctionality and allosteric site identification for design of potential wildtype reactivators. Protein J. 2018;37(3):216-222. [CrossRef]
15. Eng MY, Luczak SE, Wall TL. ALDH2, ADH1B, and ADH1C genotypes in Asians: a literature review. Alcohol Res Health. 2007;30(1):22-27.
16. Mao N, Nie S, Hong B, Li C, Shen X, Xiong T. Association between alcohol dehydrogenase-2 gene polymorphism and esophageal cancer risk: a meta-analysis. World J Surg Oncol. 2016;14(1):191. [CrossRef]
17. Avinçsal MO, Shinomiya H, Teshima M, et al. Impact of alcohol dehydrogenase-aldehyde dehydrogenase polymorphism on clinical outcome in patients with hypopharyngeal cancer. Head Neck. 2018;40(4):770-777. [CrossRef]
18. Fang F, Pan J, Su GH, et al. Association between alcohol dehydrogenase 1C gene *1/*2 polymorphism and pancreatitis risk: a meta-analysis. Genet Mol Res. 2015;14(4):15267-15275. [CrossRef]
19. Prasad B, Bhatt DK, Johnson K, et al. Abundance of Phase 1 and 2 drug-metabolizing enzymes in alcoholic and hepatitis C cirrhotic livers: a quantitative targeted proteomics study. Drug Metab Dispos. 2018;46(7):943-952. [CrossRef]
20. Huang PH, Hu CC, Chien CH, et al. The defective allele of aldehyde dehydrogenase 2 gene is associated with favorable postoperative prognosis in hepatocellular carcinoma. J Cancer. 2019;10(23):5735-5743. [CrossRef]
21. Lai CL, Yao CT, Chau GY, et al. Dominance of the inactive Asian variant over activity and protein contents of mitochondrial aldehyde dehydrogenase 2 in human liver. Alcohol Clin Exp Res. 2014;38(1):44-50. [CrossRef]
22. Zhou J, Wen Q, Li SF, et al. Significant change of cytochrome P450s activities in patients with hepatocellular carcinoma. Oncotarget. 2016;7(31):50612-50623. [CrossRef]
23. Gao J, Wang Z, Wang GJ, et al. Higher CYP2E1 activity correlates with hepatocarcinogenesis induced by diethylnitrosamine. J Pharmacol Exp Ther. 2018;365(2):398-407. [CrossRef]
24. Skurský L, Kovár J, Stachová M. A sensitive photometric assay for alcohol dehydrogenase activity in blood serum. Anal Biochem. 1979;99(1):65-71. [CrossRef]
25. Wierzchowski J, Dafeldecker WP, Holmquist B, Vallee BL. Fluorometric assays for isozymes of human alcohol dehydrogenase. Anal Biochem. 1989;178(1):57-62. [CrossRef]
26. Jiang Y, Sun A, Zhao Y, et al. Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. Nature. 2019;567(7747):257-261. [CrossRef]
27. Ma L, Lu ZN. Role of ADH1B rs1229984 and ALDH2 rs671 gene polymorphisms in the development of Alzheimer's disease. Genet Mol Res. 2016;15(4). [CrossRef]
28. Paget S. The distribution of secondary growths in cancer of the breast. Cancer Metastasis Rev. 1989;8(2):98-101. [CrossRef]
29. Li D, Zhao H, Gelernter J. Strong protective effect of the aldehyde dehydrogenase gene (ALDH2) 504lys (*2) allele against alcoholism and alcohol-induced medical diseases in Asians. Hum Genet. 2012;131(5):725-737. [CrossRef]
30. Rivera-Meza M, Quintanilla ME, Tampier L, Mura CV, Sapag A, Israel Y. Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: development of an animal model. FASEB J. 2010;24(1):266-274. [CrossRef]
31. Neumark YD, Friedlander Y, Durst R, et al. Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. Alcohol Clin Exp Res. 2004;28(1):10-14. [CrossRef]
32. Marshall VJ, Ramchandani VA, Kalu N, et al. Evaluation of the influence of alcohol dehydrogenase polymorphisms on alcohol elimination rates in African Americans. Alcohol Clin Exp Res. 2014;38(1):51-59. [CrossRef]
33. Peng GS, Wang MF, Chen CY, et al. Involvement of acetaldehyde for full protection against alcoholism by homozygosity of the variant allele of mitochondrial aldehyde dehydrogenase gene in Asians. Pharmacogenetics. 1999;9(4):463-476.
34. Peng GS, Chen YC, Tsao TP, Wang MF, Yin SJ. Pharmacokinetic and pharmacodynamic basis for partial protection against alcoholism in Asians, heterozygous for the variant ALDH2*2 gene allele. Pharmacogenet Genomics. 2007;17(10):845-855. [CrossRef]
35. Enomoto N, Takase S, Yasuhara M, Takada A. Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. Alcohol Clin Exp Res. 1991;15(1):141-144. [CrossRef]