

Distinct prognostic values of alcohol dehydrogenase mRNA expression in pancreatic adenocarcinoma

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Background: Alcohol dehydrogenase (ADH) isoenzymes have been reported as a potential diagnostic marker for pancreatic cancer, but their prognostic value in pancreatic cancer remains unclear. The aim of this investigation was to identify the prognostic value of ADH genes in human patients with pancreatic adenocarcinoma (PAAD).

Materials and methods: An RNA sequencing dataset and corresponding survival profiles of PAAD were obtained from The Cancer Genome Atlas. Survival analysis and gene set enrichment analysis were used to investigate the prediction value and potential mechanism of ADH genes in PAAD prognosis.

Results: Survival analysis of ADH genes suggests that a high expression of *ADH1A* (adjusted $P=0.037$, adjusted hazard ratio [HR] =0.627, 95% CI =0.404–0.972) and *ADH6* (adjusted $P=0.018$, adjusted HR =0.588, 95% CI =0.378–0.914) were associated with a significantly decreased risk of death, while a high expression of *ADH5* was associated with a significantly increased risk of death (adjusted $P=0.043$, adjusted HR =1.564, 95% CI =1.013–2.414). Joint effects analysis of three ADH gene prognostic markers suggests that the prognosis difference for any marker combination was more significant than that for any individual marker. The potential mechanism of *ADH1A* and *ADH6* in PAAD prognosis was that a high expression of *ADH1A* and *ADH6* was involved in the P450 pathway and biological processes, while high *ADH5* expression was involved in transforming growth factor β regulation-related pathways and biological processes, Wnt, the cell cycle, ErbB, and mitogen-activated protein kinase signaling pathways.

Conclusion: Our data suggest that *ADH1A*, *ADH5*, and *ADH6* expression may be potential prognostic markers of PAAD and in combination have a strong interaction and better predictive value for PAAD prognosis.

Keywords: prognostic, alcohol dehydrogenase, pancreatic adenocarcinoma, TCGA, GSEA

Introduction

Pancreatic cancer presents as highly lethal malignant tumors, for which mortality closely parallels incidence, with an estimated 330,400 deaths occurring worldwide in 2012.^{1,2} It is estimated that about 79,400 Chinese will die from pancreatic cancer in 2015, and it is predicted that there will be about 90,100 newly diagnosed pancreatic cancer cases in China in 2015.³ The age-standardized mortality rates of pancreatic cancer in the Chinese male population have been shown to have an upward trend.³ Pancreatic cancer patients always have a relatively poor prognosis in China with an age-standardized 5-year relative survival rate of 11.7%.⁴ Therefore, it is very important to find markers and prognostic predictive indicators that detect malignant cell transformation at an early stage.

Studies have demonstrated that alcohol dehydrogenase (ADH) is present in the pancreatic tissue and plays an important role in multiple biological processes of the pancreas.^{5,6} A study by Jelski et al reported that class III ADH activity was markedly higher in pancreatic cancer tissue than in healthy tissue.⁷ ADH isoenzymes have also been reported as a potential diagnostic marker for pancreatic cancer, and the combination of circulating ADH and macrophage inhibitory cytokine to carbohydrate antigen 19–9 can improve the overall quality of diagnosis for this lethal disease.^{8,9} Although the diagnostic value of ADH in pancreatic cancer has been identified, the prognostic value of ADH in pancreatic cancer remains unclear. The aim of this investigation was to identify the prognostic value of ADH gene expression in pancreatic cancer patients.

The Cancer Genome Atlas (TCGA) has generated comprehensive, multidimensional maps of the key genomic changes in 33 types of cancer including pancreatic adenocarcinoma (PAAD), which is available as open access. In the present study, we utilized the TCGA database to investigate the prognostic prediction value and potential mechanism of ADH genes in patients with PAAD.

Materials and methods

RNA sequencing dataset

An RNA sequencing dataset including 177 PAAD patient transcriptome and corresponding survival profiles was obtained from the TCGA web server (<https://portal.gdc.cancer.gov/>, accessed March 20, 2017). RNA sequencing datasets of 177 tumor tissues and four adjacent normal tissues were downloaded from the TCGA database; information on overall survival (OS), as well as the status of events, was available for all of these patients. Normalization of the PAAD RNA sequencing dataset was performed using DESeq, an R package for transcriptome profiling, according to the user guide.¹⁰ Genes that were 0 in >10% of all subjects were eliminated.

Association analysis

The comparison of ADH gene expression between pancreatic tumor tissue and adjacent normal tissues was done by an analysis using Metabolic gEne Rapid Visualizer¹¹ (<http://merav.wi.mit.edu/>) and TCGA dataset, respectively. Pearson correlation coefficient was used to evaluate correlations among genes in coexpression analysis.

Survival analysis

All patients were divided into two groups according to gene expression levels in tumor tissue for survival analysis.

The high-expression group consisted of patients in which gene expression levels were above the median value, and a low-expression group comprised the remaining patients. We also stratified the analysis on the basis of associations between gene expression and clinical features in OS. Alcohol history and tumor stage were adjusted in multivariate Cox proportion hazard regression analysis. On the basis of the results of coexpression analysis, we also investigated the joint effects of significant prognostic-related ADH genes in PAAD.

Gene set enrichment analysis

Differences of pathways and biological process in transcriptome levels between high and low ADH genes expression were analyzed using gene set enrichment analysis (GSEA) v2–2.2.3,^{12,13} with reference to gene sets from the Molecular Signatures Database (MSigDB) of c2 (KEGG gene sets: c2.cp.kegg.v5.2.symbols.gmt) and c5 (GO gene sets: c5.bp.v5.2.symbols.gmt, c5.cc.v5.2.symbols.gmt, and c5.mf.v5.2.symbols.gmt), respectively. The number of permutations was set at 1,000. Enrichment results satisfying a nominal *P*-value <0.05 and a false discovery rate (FDR) <0.25 were considered statistically significant.

Statistical analysis

The mRNA expression of ADH genes in tumor and adjacent nontumor tissue was analyzed using an independent sample *t*-test. The Pearson correlation coefficient was used to assess the coexpression correlation at the mRNA level, and the coexpression heat map was constructed by the corplot package in the R platform. 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% CI in uni- and multivariate analyses. The FDR in GSEA was adjusted for multiple testing with the Benjamini–Hochberg procedure to control FDR.^{14,15} Kaplan–Meier survival curves were plotted using GraphPad Prism 6.0. A value of *P*<0.05 was considered statistically significant. Data were analyzed with the help of SPSS v.20.0 software (IBM, Chicago, IL, USA).

Results

Data processing

Seven ADH genes were available in the TCGA PAAD mRNA expression dataset, *ADH1A*, *ADH1B*, *ADH1C*, *ADH4*, *ADH5*, *ADH6*, and *ADH7*. After normalization of the RNA sequencing data, we found that the expression level

of *ADH7* was very low in PAAD tumor tissue. Therefore, *ADH7* data were excluded from the present study. After normalization by DESeq, 177 pancreatic tumor tissues and 4 adjacent normal tissues expression data with six ADH genes were used for further analysis.

Association analysis

Distribution of ADH gene expression between pancreatic tumor tissue and adjacent normal tissues using MERAV (Figure 1A–F) showed that *ADH1A* and *ADH1B* were markedly downregulated in pancreatic tumor tissue (Figure 1A and B), whereas *ADH5* was significantly upregulated in tumor tissue (Figure 1E). Similar results were also observed in *ADH1B* and *ADH5* of patients with TCGA PAAD (Figure 2A), but the difference did not reach statistical significance among these genes. Coexpression analysis of ADH

genes indicated that *ADH1A* ($r=0.581$, $P<0.001$, Figure 2B), *ADH1C* ($r=0.371$, $P<0.001$, Figure 2B), and *ADH6* ($r=0.502$, $P<0.001$, Figure 2B) had a significantly positive correlation with *ADH4* in PAAD tumor tissue, while *ADH1A* ($r=0.761$, $P<0.001$, Figure 2B) and *ADH5* ($r=0.176$, $P=0.019$, Figure 2B) had a significantly positive correlation with *ADH6*.

Survival analysis

The clinical characteristics of PAAD are summarized in Table 1. Information only with regard to age, sex, alcohol history, tumor stage, and clinical outcomes of the PAAD can be obtained from the TCGA website. OS stratified by the clinical characteristics indicate that advanced tumor stage was associated with a significantly increased risk of death in PAAD patients. Survival analysis of ADH genes are showed in Figure 3A–F, and suggested that a high expression

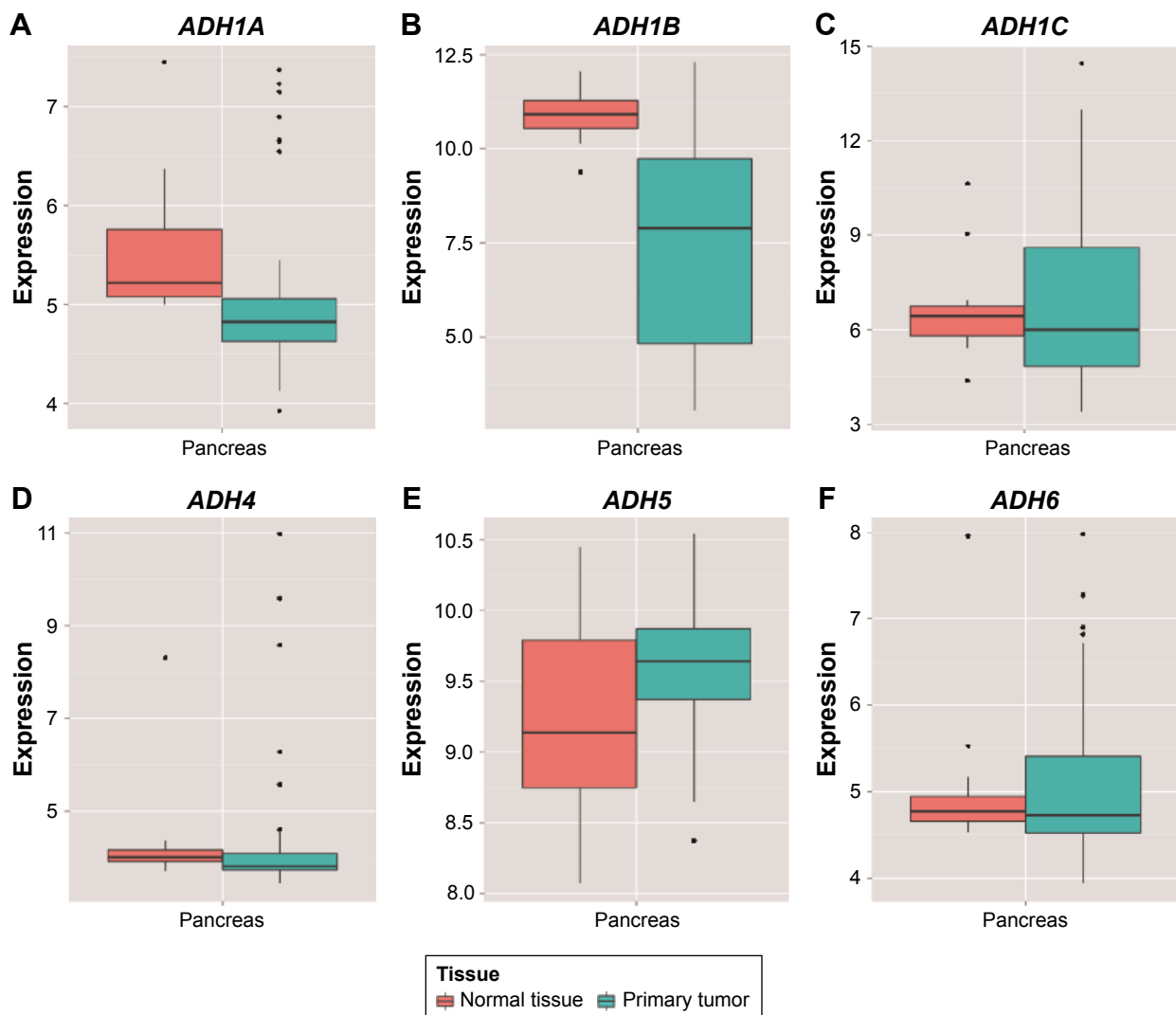


Figure 1 Distribution of ADH genes expression between pancreatic tumor tissue and adjacent normal tissues using the MERAV web server.

Notes: The order in (A–F) shows the distribution of *ADH1A*, *ADH1B*, *ADH1C*, *ADH4*, *ADH5*, and *ADH6* gene mRNA expression between pancreatic tumor tissue and adjacent normal tissues, respectively.

Abbreviations: ADH, alcohol dehydrogenase; MERAV, metabolic gEne RAPid visualizer.

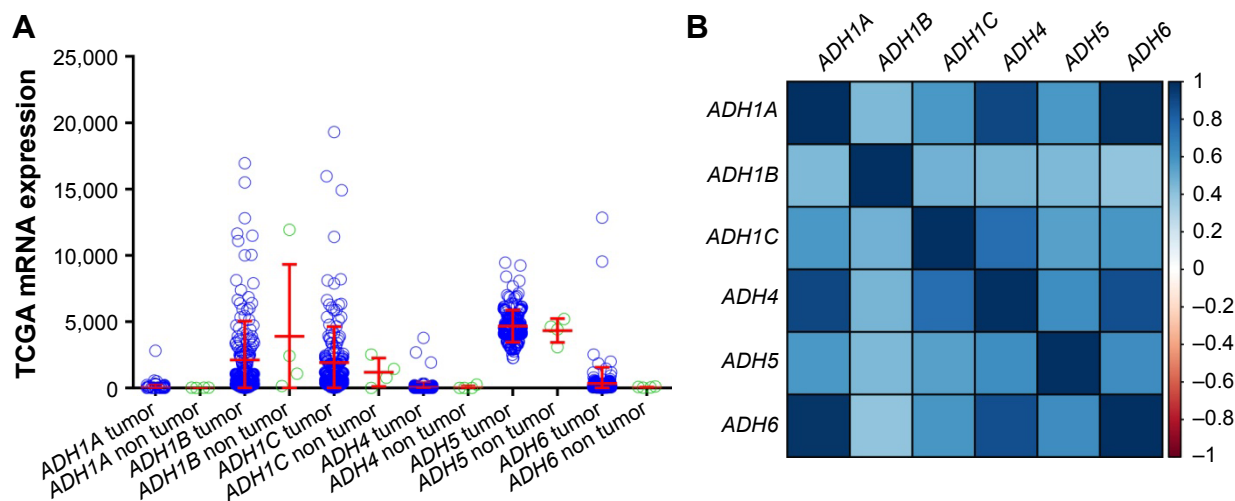


Figure 2 Gene expression distribution and coexpression heat map of ADH genes using the TCGA dataset.

Notes: (A) Distribution of ADH genes expression between the tumor tissue and adjacent normal tissues; (B) coexpression heat map of ADH genes in PAAD tumor tissue.

Abbreviations: ADH, alcohol dehydrogenase; TCGA, The Cancer Genome Atlas; PAAD, pancreatic adenocarcinoma.

of *ADH1A* (adjusted $P=0.037$, adjusted HR =0.627, 95% CI =0.404–0.972; Table 2) and *ADH6* (adjusted $P=0.018$, adjusted HR =0.588, 95% CI =0.378–0.914; Table 2) was significantly associated with a decreased risk of death and a long median survival time (MST; 545 vs 913 days for low *ADH1A* vs high *ADH1A*, log-rank $P=0.073$, Figure 3A; 592 vs 691 days for low *ADH6* vs high *ADH6*, log-rank $P=0.03$, Figure 3C; respectively) in PAAD patients, after adjusting for alcohol history and tumor stage. In contrast, a high expression of *ADH5* was significantly associated with a poor clinical outcome (MST: 702 vs 511 days for low *ADH5* vs high *ADH5*, log-rank $P=0.0079$, Figure 3B)

and an increased risk of death (adjusted $P=0.043$, adjusted HR =1.564, 95% CI =1.013–2.414; Table 2). The associations between other ADH genes and PAAD OS did not show statistical significance.

Stratification analysis

Results of the stratified analysis of *ADH1A*, *ADH5*, and *ADH6* with OS in different strata of clinical characteristics are shown in Table 3. A high expression of *ADH1A* (adjusted $P=0.017$, adjusted HR =0.468, 95% CI =0.251–0.873) and *ADH6* (adjusted $P=0.026$, adjusted HR =0.498, 95% CI =0.270–0.918) has a protective effect in male PAAD patients, while high *ADH5* expression significantly increases the risk of death (adjusted $P=0.028$, adjusted HR =1.977, 95% CI =1.075–3.636). Similar protective effects can also be found with age >60 years (adjusted $P=0.006$, adjusted HR =0.488, 95% CI =0.293–0.811) and tumor stage II (adjusted $P=0.049$, adjusted HR =0.627, 95% CI =0.394–0.997) in patients with high *ADH6* expression. However, high *ADH5* expression also increased the risk of death in patients without a history of alcohol (adjusted $P=0.014$, adjusted HR =2.574, 95% CI =1.213–5.464).

Joint effects analysis

Coexpression analysis indicates that *ADH1A* and *ADH5* were positively correlated with *ADH6* at the mRNA expression level. We further investigated the joint effects of these genes in the prediction of PAAD prognosis. The combination of *ADH1A* and *ADH5* was divided into three groups (Table S1) for assessing the prognostic value in PAAD according to the associations between the genes and OS. Similar

Table 1 Clinical characteristics of PAAD patients

Variables	Events/total	MST (days)	Crude HR (95% CI)	Log-rank P-value
Age (years) ^a				
≤60	25/54	684	I	0.132
>60	67/123	598	1.421 (0.897–2.251)	
Sex				
Male	46/97	661	I	0.343
Female	46/80	568	1.219 (0.809–1.836)	
Alcohol history ^b				
No	32/64	607	I	0.599
Yes	52/101	603	1.126 (0.724–1.749)	
Tumor stage ^c				
I	7/21	NA	I	0.033
II	82/146	596	2.333 (1.069–5.089)	
III+IV	3/7	545	1.715 (0.438–6.719)	

Notes: ^aAge at initial pathologic diagnosis; ^binformation of alcohol history was unavailable in 12 patients; ^cinformation of tumor stage was unavailable in 3 patients.

Abbreviations: HR, hazard ratio; MST, median survival time; PAAD, pancreatic adenocarcinoma; NA, not available.

Table 2 Associations between ADH genes and OS in PAAD patients

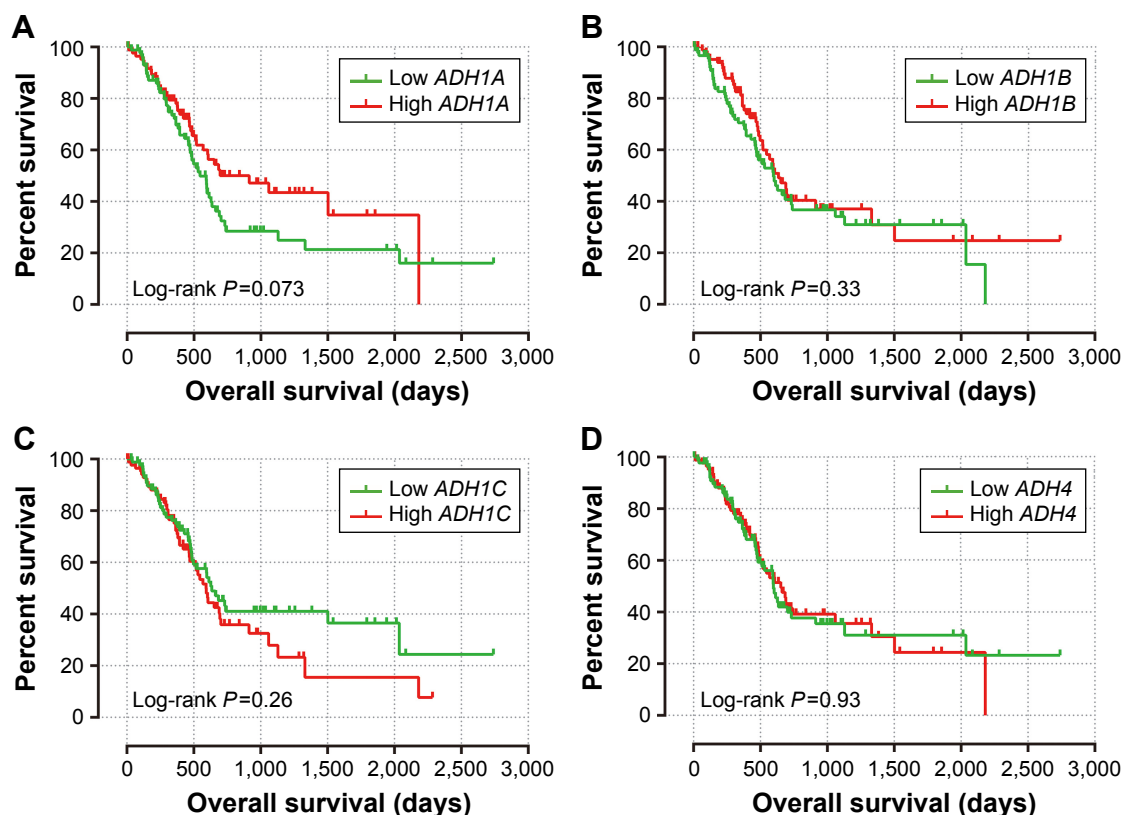
Gene expression	Events/total	MST (days)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value ^a
<i>ADH1A</i>						
Low	54/89	545	1		1	
High	38/88	913	0.685 (0.452–1.039)	0.075	0.627 (0.404–0.972)	0.037
<i>ADH1B</i>						
Low	51/89	598	1		1	
High	41/88	634	0.816 (0.540–1.232)	0.333	0.858 (0.552–1.334)	0.497
<i>ADH1C</i>						
Low	44/89	634	1		1	
High	48/88	592	1.265 (0.838–1.909)	0.263	1.038 (0.656–1.641)	0.874
<i>ADH4</i>						
Low	47/89	598	1		1	
High	45/88	652	0.981 (0.651–1.478)	0.926	0.917 (0.592–1.421)	0.698
<i>ADH5</i>						
Low	40/89	702	1		1	
High	52/88	511	1.760 (1.153–2.685)	0.009	1.564 (1.013–2.414)	0.043
<i>ADH6</i>						
Low	53/89	592	1		1	
High	39/88	691	0.634 (0.419–0.961)	0.032	0.588 (0.378–0.914)	0.018

Note: ^aAdjusted for alcohol history and tumor stage.

Abbreviations: ADH, alcohol dehydrogenase; HR, hazard ratio; MST, median survival time; OS, overall survival; PAAD, pancreatic adenocarcinoma.

classification methods were also used for the combinations of *ADH1A*+*ADH6*, *ADH5*+*ADH6*, and *ADH1A*+*ADH5*+*ADH6*, and the detailed grouping information is shown in [Table S1](#). Joint effects analysis in the combination of *ADH1A* and

ADH5 demonstrated that group 2 (adjusted $P=0.011$, adjusted HR =0.525, 95% CI =0.320–0.862, Table 4; Figure 4A) and group 3 (adjusted $P=0.003$, adjusted HR =0.379, 95% CI =0.198–0.723, Table 4; Figure 4A) were associated with

**Figure 3** (Continued)

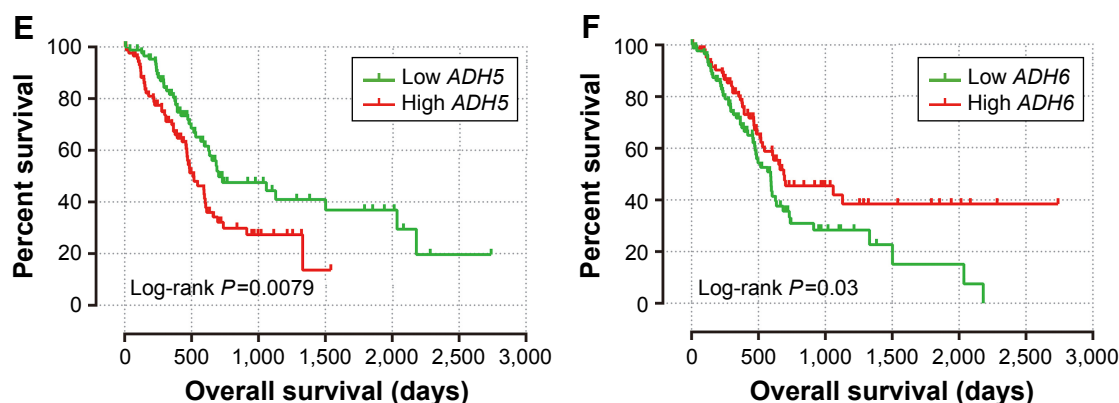


Figure 3 The prognostic value of ADH genes expression in PAAD patients.

Notes: (A) OS stratified by *ADH1A*; (B) OS stratified by *ADH1B*; (C) OS stratified by *ADH1C*; (D) OS stratified by *ADH4*; (E) OS stratified by *ADH5*; (F) OS stratified by *ADH6*.

Abbreviations: ADH, alcohol dehydrogenase; PAAD, pancreatic adenocarcinoma; OS, overall survival.

a significantly decreased risk of death in PAAD, compared to group 1. In Table 4, a protective effect can also be observed in the combination of *ADH1A*+*ADH6* (group ii vs group i, adjusted $P=0.029$, adjusted HR =0.576, 95% CI =0.351–0.946; group iii vs group i, adjusted $P=0.003$, adjusted HR =0.408, 95% CI =0.225–0.741, Figure 4B), *ADH5*+*ADH6* (group C vs group A, adjusted $P=0.004$, adjusted HR =0.378, 95% CI =0.196–0.727, Figure 4C) and *ADH1A*+*ADH5*+*ADH6* (group III vs group I, adjusted $P=0.014$, adjusted HR =0.475, 95% CI =0.262–0.861; group IV vs group I, adjusted $P=0.003$, adjusted HR =0.221, 95% CI =0.082–0.597, Figure 4D), respectively. Our joint effects analysis of the three ADH gene prognostic markers suggests that the prognosis difference for any marker combination was more significant than that for any individual marker.

Gene set enrichment analysis

To further explore the potential mechanism of ADH genes in PAAD prognosis, we used the PAAD genome-wide RNA sequencing dataset for GSEA. GSEA results of the c5 reference gene set suggest that high *ADH1A* expression was involved in the epoxygenase P450 pathway and cell differentiation regulation biological processes (Figure 5A and B; Table S2), while the enrichment of c2 indicates that high *ADH1A* is also involved in drug metabolism, cytochrome P450, fatty acid metabolism, and peroxisome proliferator-activated receptor signaling pathway (Figure 5C–F; Table S3). GSEA results reveal that the high expression of *ADH5* was correlated to the transforming growth factor β (TGF- β) regulation-related biological process (Figure 6A–E; Table S4), whereas the c2 enrichments suggest that high *ADH5* expression is involved in TGF- β , Wnt, cell cycle, ErbB, mitogen-activated protein

Table 3 Stratified analysis of ADH genes and OS in PAAD patients

Variables	<i>ADH1A</i>				<i>ADH5</i>				<i>ADH6</i>			
	Low	High	Adjusted HR (95% CI)	Adjusted P-value ^a	Low	High	Adjusted HR (95% CI)	Adjusted P-value ^a	Low	High	Adjusted HR (95% CI)	Adjusted P-value ^a
Age (years) ^b												
≤60	23	31	0.474 (0.194–1.155)	0.100	25	29	1.521 (0.628–3.683)	0.352	31	23	0.744 (0.292–1.896)	0.535
>60	66	57	0.787 (0.470–1.317)	0.362	64	59	1.638 (0.989–2.713)	0.055	58	65	0.488 (0.293–0.811)	0.006
Sex												
Male	46	51	0.468 (0.251–0.873)	0.017	54	43	1.977 (1.075–3.636)	0.028	45	52	0.498 (0.270–0.918)	0.026
Female	43	37	0.919 (0.489–1.727)	0.792	35	45	1.039 (0.549–1.968)	0.906	44	36	0.806 (0.422–1.539)	0.514
Alcohol history ^c												
No	34	30	0.677 (0.327–1.403)	0.295	30	34	2.574 (1.213–5.464)	0.014	28	36	0.617 (0.302–1.259)	0.184
Yes	49	52	0.598 (0.343–1.043)	0.070	53	48	1.147 (0.663–1.984)	0.624	53	48	0.587 (0.333–1.034)	0.065
Tumor stage ^d												
I	13	8	0.248 (0.030–2.074)	0.198	11	10	1.588 (0.352–7.160)	0.547	10	11	0.444 (0.086–2.296)	0.333
II	71	75	0.680 (0.428–1.079)	0.101	74	72	1.583 (0.998–2.511)	0.051	76	70	0.627 (0.394–0.997)	0.049
III+IV	3	4	0.331 (0.021–5.355)	0.437	2	5	1.0 (0.084–11.894)	1.0	2	5	0.331 (0.021–5.355)	0.437

Notes: ^aAdjusted for alcohol history and tumor stage; ^bage at initial pathologic diagnosis; ^cinformation of alcohol history was unavailable in 12 patients; ^dinformation of tumor stage was unavailable in 3 patients.

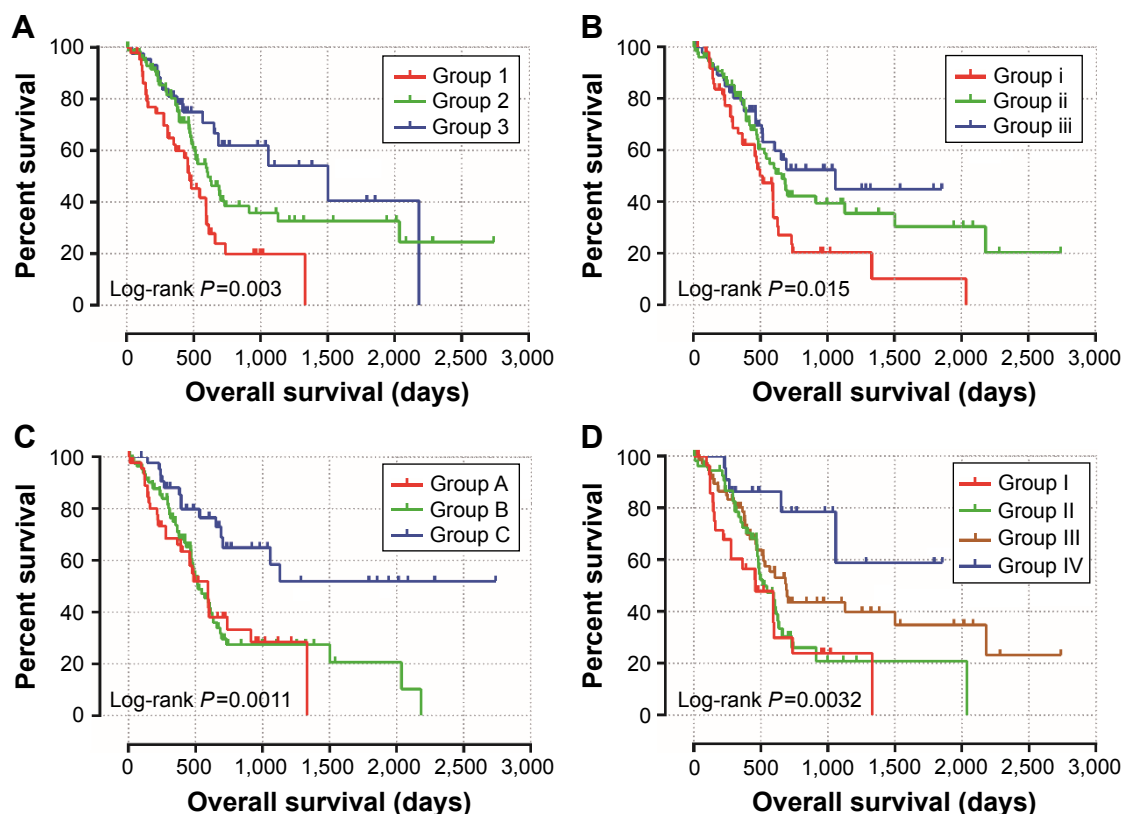
Abbreviations: ADH, alcohol dehydrogenase; HR, hazard ratio; MST, median survival time; OS, overall survival; PAAD, pancreatic adenocarcinoma.

Table 4 Joint effects analysis of ADH genes and OS in PAAD patients

Groups	Events/total	MST (days)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value ^a
ADH1A+ADH5						
Group 1	30/45	470	1		1	
Group 2	46/87	607	0.556 (0.349–0.887)	0.014	0.525 (0.320–0.862)	0.011
Group 3	16/45	1,502	0.378 (0.205–0.697)	0.002	0.379 (0.198–0.723)	0.003
ADH1A+ADH6						
Group i	34/50	498	1		1	
Group ii	39/78	661	0.592 (0.371–0.944)	0.028	0.576 (0.351–0.946)	0.029
Group iii	19/49	1,059	0.473 (0.269–0.832)	0.009	0.408 (0.225–0.741)	0.003
ADH5+ADH6						
Group A	27/47	592	1		1	
Group B	51/83	518	0.936 (0.585–1.496)	0.782	0.904 (0.552–1.478)	0.687
Group C	14/47	NA	0.339 (0.176–0.654)	0.001	0.378 (0.196–0.727)	0.004
ADH1A+ADH5+ADH6						
Group I	19/29	460	1		1	
Group II	34/55	517	0.761 (0.433–1.336)	0.342	0.726 (0.403–1.308)	0.286
Group III	34/69	691	0.503 (0.284–0.890)	0.018	0.475 (0.262–0.861)	0.014
Group IV	5/24	NA	0.212 (0.079–0.569)	0.002	0.221 (0.082–0.597)	0.003

Notes: Group I: low ADH1A+high ADH5; Group 2: low ADH1A+low ADH5 or high ADH1A+high ADH5; Group 3: high ADH1A+low ADH5; Group i: low ADH1A+low ADH6; Group ii: low ADH1A+high ADH6 or high ADH1A+low ADH6; Group iii: high ADH1A+high ADH6; Group A: high ADH5+low ADH6; Group B: low ADH5+low ADH6 or high ADH5+high ADH6; Group C: low ADH5+high ADH6; Group I: low ADH1A+high ADH5+high ADH6; Group II: low ADH1A+high ADH5+low ADH6 or low ADH1A+low ADH5+low ADH6 or high ADH1A+high ADH5+low ADH6; Group III: low ADH1A+low ADH5+high ADH6 or high ADH1A+high ADH5+high ADH6 or high ADH1A+low ADH5+low ADH6; Group IV: high ADH1A+low ADH5+high ADH6; ^aAdjusted for alcohol history and tumor stage.

Abbreviations: ADH, alcohol dehydrogenase; HR, hazard ratio; MST, median survival time; OS, overall survival; PAAD, pancreatic adenocarcinoma; NA, not available.

**Figure 4** Kaplan–Meier survival curve for joint effects analysis among ADH1A, ADH5, and ADH6 in PAAD patients.

Notes: (A) Joint effects analysis of ADH1A and ADH5; (B) joint effects analysis of ADH1A and ADH6; (C) joint effects analysis of ADH5 and ADH6; (D) joint effects analysis of ADH1A, ADH5, and ADH6.

Abbreviations: ADH, alcohol dehydrogenase; PAAD, pancreatic adenocarcinoma.

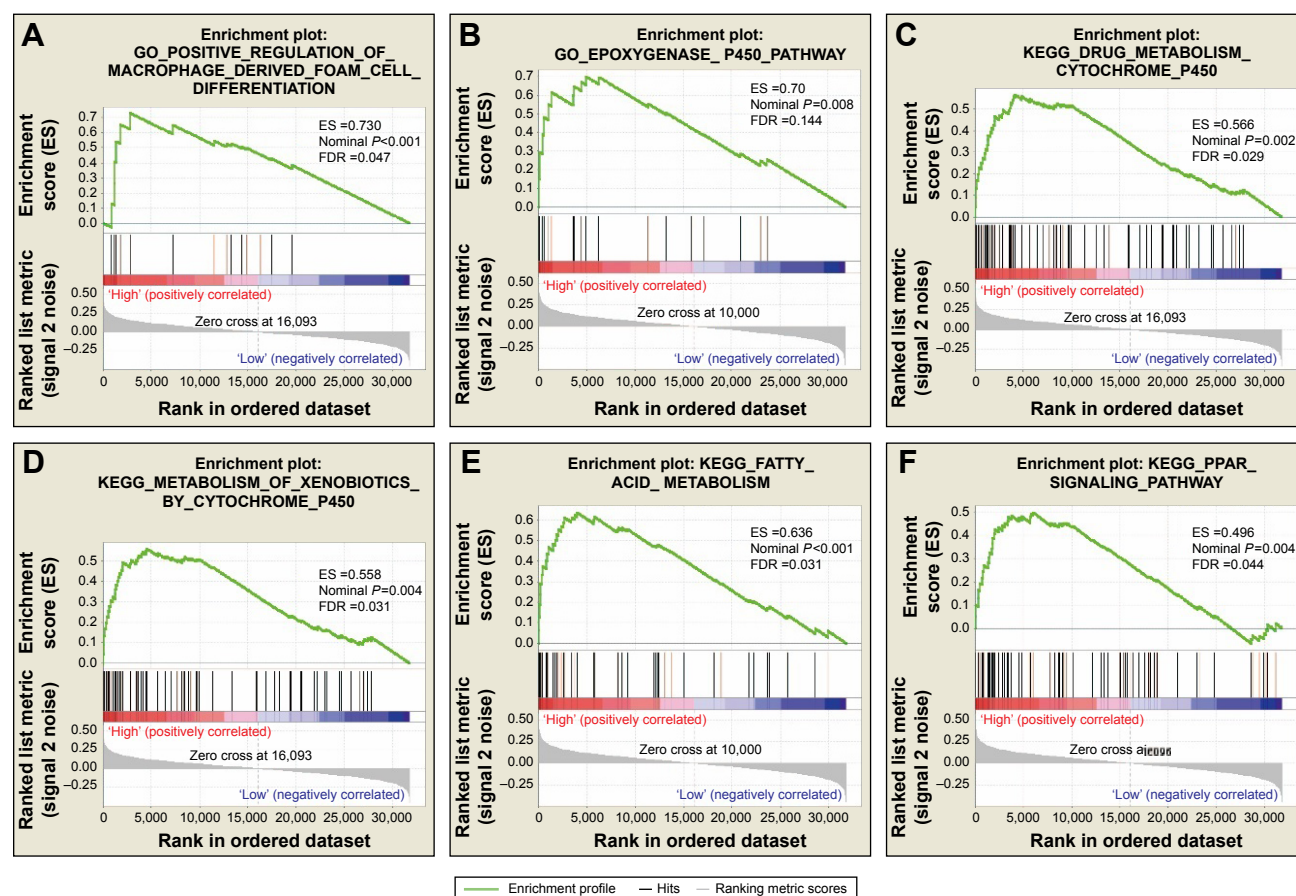


Figure 5 GSEA results of *ADH1A* expressed in PAAD patients.

Notes: (A and B) GSEA results of c5 reference gene sets for high *ADH1A* expression groups; (C–F) GSEA results of c2 reference gene sets for high *ADH1A* expression groups.

Abbreviations: ADH, alcohol dehydrogenase; ES, enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; PAAD, pancreatic adenocarcinoma.

kinase (MAPK), and the pancreatic cancer signaling pathway (Figure 6F–I; Table S5). We also investigated the potential mechanism in *ADH6* and demonstrated that high *ADH6* expression was related to primary alcohol metabolic processes (Figure 7A; Table S6), fatty acid and retinol metabolism, and drug metabolism cytochrome P450 (Figure 7B–F; Table S7).

Discussion

Ethanol is first metabolized by ADH isoenzymes and then the resulting product is further metabolized by aldehyde dehydrogenase (ALDH) isoenzymes into acetic acid.¹⁶ The disproportion between ADH and ALDH may lead to an increased ability for ethanol oxidation and less capability to remove acetaldehyde, resulting in deleterious alcohol metabolites accumulating in vivo, which may subsequently cause oncogenesis.¹⁷ ADH isoenzymes are divided into several classes on the basis of differences in biological

characteristics.¹⁸ Isoenzymes of class I ADH are encoded by *ADH1A*, *ADH1B*, and *ADH1C*, whereas class II, III, IV, and class V ADH are encoded by *ADH4*, *ADH5*, *ADH6*, and *ADH7*, respectively.¹⁹ Numerous studies have shown that the serum levels of class I ADH are a potential diagnostic marker in multiple cancers, including renal cell,²⁰ brain,²¹ colorectal,²² endometrial,²³ and cervical cancers.²⁴ Similar diagnostic values can also be observed in class III ADH for pancreatic cancer,⁸ and class IV ADH for esophageal²⁵ and gastric cancers.²⁶ The diagnostic values of ADH isoenzymes in other cancers have not yet been reported, but the difference of ADH isoenzymes between cancer patients and healthy subjects has already been observed. Both the serum and tissue expression level of class I and total ADH were significantly increased in liver,^{27,28} colorectal,^{29,30} and brain cancers,^{31,32} while ALDH levels were not statistically significant different between cancer patients and healthy subjects. The upregulation trend of class I ADH between

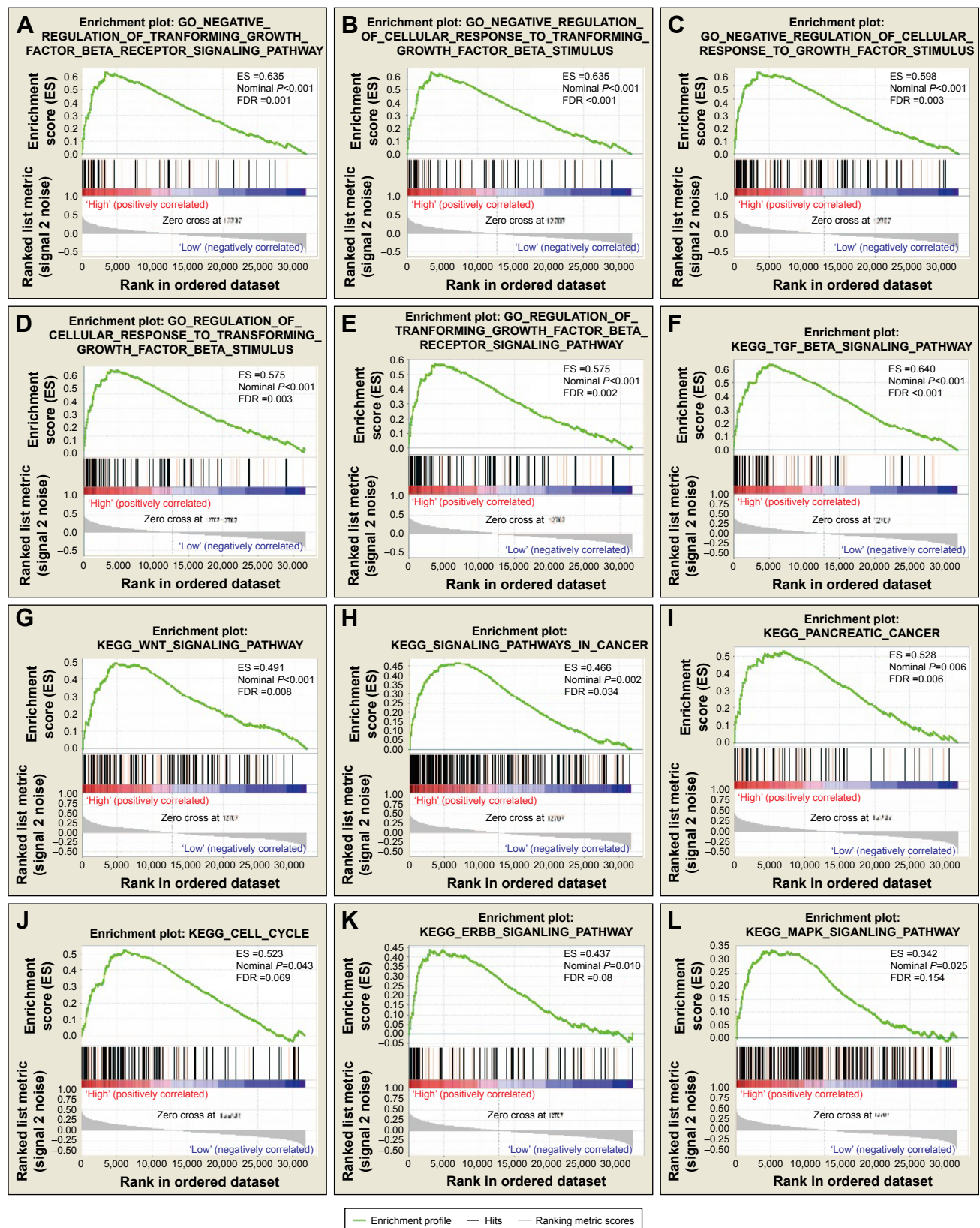


Figure 6 GSEA results of *ADH5* expressed in PAAD patients.

Notes: (A–E) GSEA results of c5 reference gene sets for high *ADH5* expression groups; (F–L) GSEA results of c2 reference gene sets for high *ADH5* expression groups.

Abbreviations: ADH, alcohol dehydrogenase; ES, enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; PAAD, pancreatic adenocarcinoma.

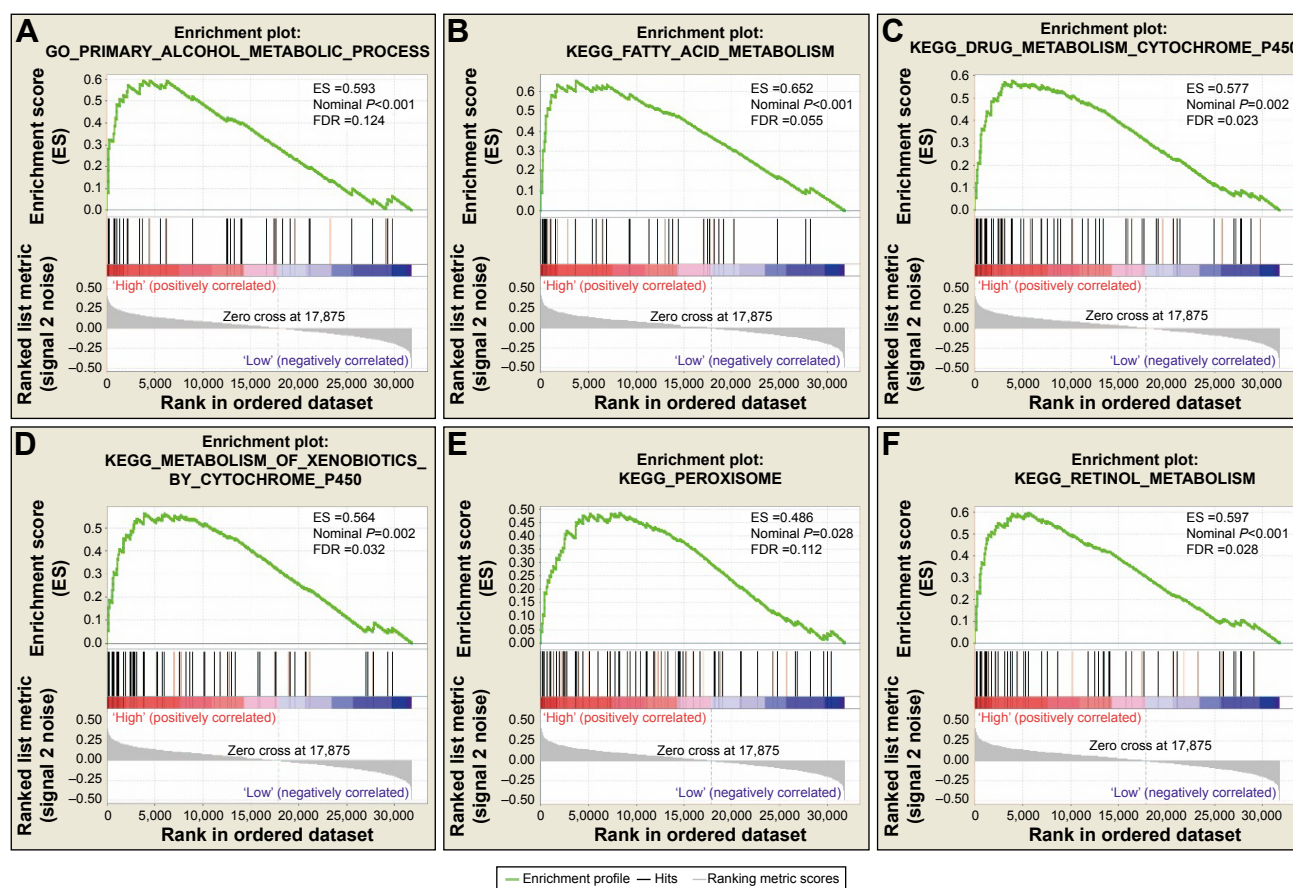


Figure 7 GSEA results of *ADH6* expressed in PAAD patients.

Notes: (A) GSEA results of c5 reference gene sets for high *ADH6* expression groups; (B–F) GSEA results of c2 reference gene sets for high *ADH6* expression groups.

Abbreviations: ADH, alcohol dehydrogenase; ES, enrichment score; FDR, false discovery rate; GSEA, gene set enrichment analysis; PAAD, pancreatic adenocarcinoma.

cancer and healthy tissue can also be found in cervical,³³ ovarian,³⁴ endometrial,³⁵ and renal cell cancers,³⁶ whereas the upregulation trend of class I ADH for cancer serum has been reported in bladder and esophageal cancers.^{37,38} However, the expression of class I ADH in breast cancer was significantly decreased compared to the tissue of healthy subjects, and the expression of ALDH was unchanged.³⁹ An upregulation trend of class III and class IV ADH was also shown in pancreatic and esophageal tumor tissue, respectively.^{7,40} Besides the use of the differences of ADH isoenzymes expression for cancer diagnosis, the genetic variant of ADH can also change the susceptibility of cancer. Polymorphisms of *ADH1C* are significantly associated with the risk of colorectal cancer,⁴¹ oral squamous cell carcinoma,^{42,43} bladder cancer,⁴⁴ upper aerodigestive tract cancers,^{45,46} head and neck squamous cell carcinoma,⁴⁷ and gastric cancer.^{48,49}

Despite numerous reports of ADH isoenzyme diagnosis values and cancer susceptibility, the prognostic value of ADH genes has rarely been investigated. A previous study by Wei et al indicated that *ADH4* may act as a potential

prognostic marker for hepatocellular carcinoma (HCC), and lower expression of *ADH4* may be associated with a worse survival.⁵⁰ Our previous study on hepatitis B virus (HBV)-related HCC also demonstrates that the upregulation of *ADH1A*, *ADH1C*, and *ADH6* in HCC tumor tissues was associated with favorable prognosis, whereas high *ADH1C* and *ADH5* reduced the risk of tumor recurrence in HBV-related HCC, respectively.⁵¹ In the current study, our results indicate that a high expression of *ADH1A* and *ADH6* has a protective effect in PAAD prognosis, while a high expression of *ADH5* may increase the risk of death. In addition, the combination of these three ADH genes has prediction values for PAAD prognosis, and the prognosis difference between different combination groups was significant. Our findings imply that individuals of these three ADH genes may serve as a PAAD prognostic marker; however, their combination showed a strong interaction and better predictive value for PAAD prognosis. Once validated, ADH genes may be valuable biomarkers in PAAD diagnosis and prognostic prediction, and later these biomarkers may be used in combination with

other clinical diagnosis and prognostic factors for decision-making in PAAD management.

To investigate the potential mechanism of ADH genes in PAAD prognosis, we used a genome-wide RNA sequencing dataset in GSEA and substantiated that both *ADH1A* and *ADH6* were involved in drug metabolism cytochrome P450 and fatty acid metabolism pathways. Human cytochrome P450 (CYP) enzymes are mainly distributed in the smooth endoplasmic reticulum and involved in detoxification through the metabolism of toxic fat-soluble substances to water-soluble substances, which are then excreted.⁵² P450 enzymes play a key role in cancer formation and cancer treatment, and mediate the metabolic activation of anticancer drugs and precarcinogens, as well as anticancer drug inactivation.⁵³ An in vitro study has substantiated that the expression of CYP2B1 enzymes (retrovirus-mediated transduction) leads to an increased susceptibility to ifosfamide in pancreatic cancer.⁵⁴ Studies suggest that P450 enzymes have the potential to be used as distinguishing markers in pancreatic pathology and targets of pancreatic cancer gene therapy.^{55,56} On the basis of GSEA results, we deduced that both *ADH1A* and a high expression of *ADH6* were involved in the P450-related pathway and biological processes that are associated with the progress and treatment of pancreatic cancer, and may play a role in OS of PAAD via P450.

TGF- β regulation-related pathways and biological processes, Wnt, the cell cycle, ErbB, and the MAPK signaling pathway were significantly enriched in the *ADH5* high-expression group. TGF- β family members participate in multiple cellular functions such as proliferation, apoptosis, differentiation, and migration. Research by Friess et al substantiated that an enhanced expression of TGF- β isoforms in pancreatic cancer was correlated with a worse survival,⁵⁷ while a study by Glazer et al demonstrated that patients with early-stage pancreatic cancer have longer median survival with TGF β 1 overexpression.⁵⁸ In addition, studies have reported that the TGF- β pathway may serve as a potential target for targeted therapy^{59,60} and the inhibition of the TGF pathway can decrease PAAD growth and invasiveness.⁶¹ These findings suggest that high *ADH5* expression may influence TGF- β regulation-related pathways and biological processes, and, therefore, may play a role in PAAD prognosis.

The Wnt signaling pathway plays an important role in physiological and pathological processes including the occurrence and development of tumors. Activation of the Wnt/ β -catenin signaling pathway may enhance pancreatic cancer development⁶² and increase pancreatic cancer

tumorigenicity via miR-744.⁶³ Moreover, Jiang et al indicated that Wnt2 expression in pancreatic cancer tissues was significantly associated with tumor development by activation of the Wnt pathways and serves as a potential candidate for targeted therapy of pancreatic cancer.⁶⁴ The ErbB signaling pathway functions through ErbB family members (including ErbB-1, ErbB-2, ErbB-3, and ErbB-4) and the MAPK pathway is a common downstream target of all ErbB receptors. Previous studies indicate that ErbB-1 and ErbB-3 play an important role in pancreatic cancer and may serve as a potential candidate for targeted therapy of pancreatic cancer.^{65–67} Furthermore, Koizumi et al revealed that it is necessary for p38 MAPK signaling activation in gemcitabine-induced cell death in pancreatic cancer, and conclude that p38 MAPK signaling pathways could serve as a novel target for gemcitabine-based therapy.⁶⁸ In summary, the potential mechanism of high *ADH5* expression in PAAD prognosis is probably because it is involved in multiple biological processes and signaling pathways that are related to pancreatic cancer development, the cell cycle, targeted therapy, and survival.

There were some limitations to our study that need to be recognized. First, the clinical information from the TCGA database was not comprehensive, and the information for PAAD patients from the TCGA, such as tumor size, histology, lymphatic invasion, venous invasion, and treatment, was not available on the TCGA website. Therefore, our study evaluated the association between ADH gene expression and OS on the basis of multivariate survival analysis that was only adjusted for alcohol history and tumor stage in a Cox proportional hazards regression model. Second, only four PAAD adjacent normal tissues expression data are available in TCGA, and this resulted in a test with low power. Therefore, further investigations of ADH gene distribution between tumors and adjacent normal tissues are needed. Third, our current study based on the TCGA database to analyze the prognosis prediction of the mRNA expression level of ADH isoforms lacks verification at protein level. Therefore, future research is still needed to address these issues.

Despite these limitations, ours is the first study to investigate the association between individual ADH gene expression and OS in PAAD patients, as well as the joint effects of prognostic values among three ADH genes. We also investigated the potential mechanism of ADH genes in PAAD prognostics using the GSEA approach. These findings provide insight into ADH genes in cancer clinical outcomes and may have clinical utility for prognosis prediction and decision-making in PAAD management.

Conclusion

Our data suggest that *ADH1A*, *ADH5*, and *ADH6* may be potential prognostic biomarkers of PAAD, and their combination showed a strong interaction and better predictive value for PAAD prognosis. The potential mechanism of *ADH1A* and *ADH6* in PAAD prognosis was that a high expression of *ADH1A* and *ADH6* was involved in the P450-related pathway and biological processes, while high *ADH5* expression was involved in the TGF- β regulation-related pathway and biological processes, Wnt, the cell cycle, ErbB, and the MAPK signaling pathway. Functional experiments will be needed for the further validation of these findings in a future study. Due to the small sample size and incomplete clinical information in the current study, further well-designed and larger sample size studies are necessary to validate our results.

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Disclosure

The authors report no conflicts of interest in this work.

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