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Expression and clinical significance of BDH1 in liver cancer

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Abstract

Liver cancer is a deadly disease with generally poor patient outcomes. BDH1 is a key enzyme that regulates the metabolism and synthesis of ketone bodies. This study sought to explore the prognostic relevance of BDH1 mRNA expression in liver cancer.

We utilized the Cancer Genome Atlas datasets to analyze the relationship between BDH1 expression and clinical outcomes. We used Kaplan–Meier curves and Cox analyses to explore the relevance of BDH1 mRNA levels to patient prognosis. Further gene set enrichment analysis was conducted as a means of comparing differences in gene expression as a function of BDH1 expression.

Liver cancer samples exhibited significantly decreased BDH1 mRNA expression, and that this downregulation was correlated with a number of clinicopathological variables including gender, histologic grade, stage, TNM classification, and both overall and relapse-free survival. We further determined that BDH1 mRNA expression was an independent predictor of liver cancer patient prognosis. A subsequent gene set enrichment analysis found genes affected by BDH1 expression to be those enriched in pathways relating to MYC and wnt/β-catenin signaling.

Our preliminary findings demonstrate for the first time that low expression of BDH1 mRNA is a potentially valuable independent prognostic indicator for liver cancer detection.

Abbreviations: BDH1 = 3-hydroxybutyrate dehydrogenase 1, GSEA = gene set enrichment analysis, MSigDB = molecular signatures database, TCGA = The cancer genome atlas.

Keywords: BDH1, gene set enrichment analysis, liver cancer, prognosis, the Cancer Genome Atlas

1. Introduction

Liver cancer remains one of the most common and deadliest forms of cancer in the world, and the second leading cause of cancer-associated mortality.[1] Following diagnosis, roughly two thirds of patients cannot be cured of their liver cancer.[2] Even when patients are able to undergo systemic anti-tumor therapy, there is a high rate of chemoresistance in liver cancers in addition to a general lack of effective prognostic or diagnostic monitoring approaches, resulting in a high rate of liver cancer recurrence and metastasis.[3,4] There is therefore an urgent need for the identification of novel prognostic molecules linked with liver cancer progression or treatment through the mining of available liver cancer datasets.

The gene BDH1 (3-hydroxybutyrate dehydrogenase 1; also known as SDR9C1), located on chromosome 3q29, encodes a protein in the short-chain dehydrogenase and reductase family. This protein is an enzyme which localizes to the mitochondria and contains two thiol groups (SH-1 and SH-2) per molecule,[5] and which requires phosphatidylcholine (PC) to mediate its effective enzymatic activity.[5,6] Once localized to the inner portion of the mitochondrial membrane, BDH1 serves as an integral membrane protein and plays important roles in modulating interactions between lipids and protein-lipid interactions at the membrane surface.[7] In the context of fatty acid catabolism, BDH1 promotes the interconversion of acetoacetate andβ-hydroxy butyrate (β-HB).[8,9] When fatty acids undergo β-oxidation, the resultant acetyl CoA undergoes conversion into acetoacetic acid (AcAc).[9,10] BDH1 then reduces AcAc in order to yield β-HB.[8,9] In mitochondria outside the liver, BDH1 can

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also reverse this process, converting β-HB back into AcAc.[11] As such, BDH1 is one of the most important rate-limiting enzymes for ketone catabolism and ketolysis.

Most research regarding BDH1 has been limited to normal organisms or to the context of metabolic disease. In addition, BDH1 was found to be highly expressed in nonalcoholic steatohepatitis liver tissue as compared with normal liver.[12] Recently, studies have shown that the expression of BDH1 mRNA is altered in abnormal rat liver cancer cells relative to normal liver cells in these animals.[13,14] This attracted our interest, as we were seeking to identify prognostic biomarkers of cancer. As such, in the present study we sought to measure BDH1 mRNA expression in liver cancer samples in order to assess how this expression is linked to key clinical parameters, and to determine how well BDH1 mRNA expression can facilitate liver cancer diagnosis and/or prognostic predictions in patients.

Table 1

BDH1 mRNA expression and clinical parameters in liver cancer patients.

Characteristics	Number of pts(%)
Age	
NA	1 (0.00)
<55	117 (31.45)
>=55	255 (68.55)
Gender	
FEMALE	121 (32.44)
MALE	252 (67.56)
histological type	
Fibrolamellar Carcinoma	3 (0.8)
Hepatocellular Carcinoma	363 (97.32)
Hepatocholangiocarcinoma (Mixed)	7 (1.88)
Histologic grade	
NA	5 (1.34)
G1	55 (14.75)
G2	178 (47.72)
G3	123 (32.98)
G4	12 (3.22)
Stage	
NA	24 (6.43)
I	172 (46.11)
II	87 (23.32)
III	85 (22.79)
IV	5 (1.34)
T_classification	
NA	2 (0.54)
T1	182 (48.79)
T2	95 (25.47)
T3	80 (21.45)
T4	13 (3.49)
TX	1 (0.27)
N_classification	
NA	1 (0.27)
N0	253 (67.83)
N1	4 (1.07)
NX	115 (30.83)
M_classification	
M0	267 (71.58)
M1	4 (1.07)
MX	102 (27.35)
Radiation_therapy	
NA	25 (6.7)
NO	340 (91.15)
YES	8 (2.14)
Residual_tumor	
NA	7 (1.88)
R0	326 (87.4)
R1	17 (4.56)
R2	1 (0.27)
RX	22 (5.9)
Vital_status	
DECEASED	130 (34.85)
LIVING	243 (65.15)
Sample type	
Primary Tumor	371 (99.46)
Recurrent Tumor	2 (0.54)
Overall survival	
NA	6 (1.6)
NO	237 (63.54)
YES	130 (34.85)
Relapse-free survival	
NA	53 (14.2)
NO	179 (48.0)
YES	141 (37.8)
BDH1	
High	147 (39.41)
Low	226 (60.59)
Type	
1	373 (100)

NA: not available.

2. Methods

2.1. Patient clinical features

We obtained TCGA-LIHC datasets including a variety of clinicopathological parameters for individual liver cancer patients from UCSC Xena, and then we further assessed BDH1 expression as in log2(x+1) transformed RSEM normalized count in the RNA-seq data associated with these samples using R (v3.5.1).[15] No ethical approval was necessary because these are from public datasets.

2.2. Statistical analyses

We used box plots to assess differences in BDH1 mRNA expression between groups of patients by ggplot2.[16] We ultimately divided patients into BDH1-high or -low groups based on criteria established using the pROC package.[17] This package was then further used to draw an ROC curve for evaluating the sensitivity and specificity of BDH1 as a means of diagnosing liver cancer. Chi-squared tests were used to assess the relationship between BDH1 expression and clinical parameters, together with Fisher exact test. Kaplan-Meier curves were used to assess differences in overall or relapse-free survival in patients as a function of BDH1 expression using a survival package in R.[18] We then identified relevant clinical variables via a univariate Cox analysis, and assessed their ability to independently predict liver cancer patient prognosis, overall survival, or relapse-free survival

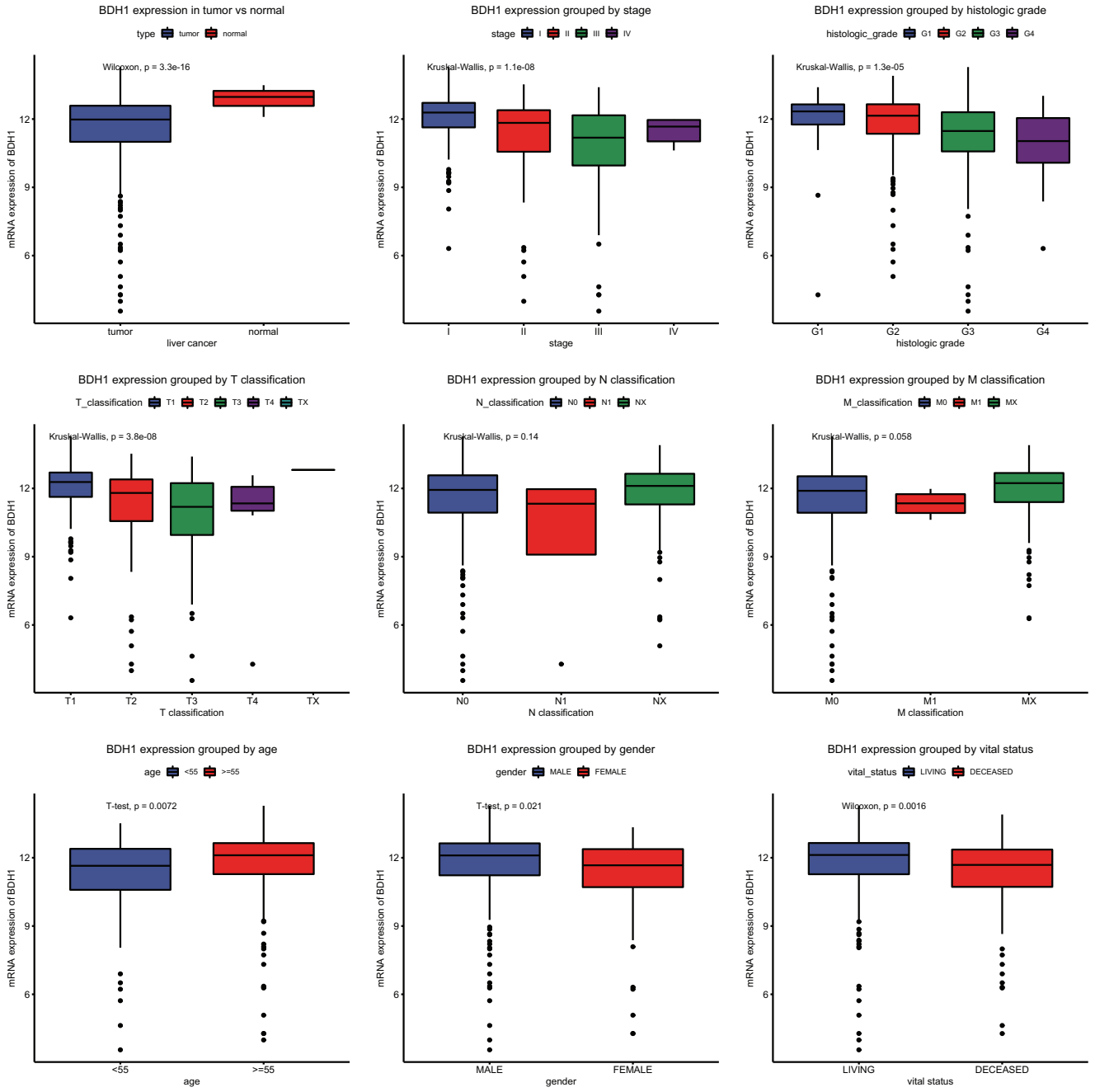

Figure 1. Assessment of the relationship between BDH1 mRNA expression and clinical parameters. Parameters assessed included patient age, gender, survival status, clinical stage, histological grade, and TNM classification. The unit of RNAseq data is log2(x+1) transformed RSEM normalized count.

via a multivariate Cox analysis. R was used for all analyses. $P < .05$ was considered statistically significant.

2.3. Gene set enrichment analysis (GSEA)

A GSEA approach was used to compare differences in gene expression between patients that were low BDH1 expressors.[19,20] We obtained the "h.all.v6.2.symbols.gmt" gene set information from the Molecular Signatures Database (http://software.broadinstitute.org/gsea/ msigdb/index.jsp). This analysis provided a summary of the biological processes linked to a given gene set, classifying these processes as appropriate. This analysis was run 1000 times in various permutations to produce a normalized enrichment score. Our GSEA analysis was conducted with a significance threshold for enrichment of $P < .05$ and a false discovery rate < 0.25. GSEA software 3.0 from the Broad Institute was used for this analysis.

3. Results

3.1. Patient clinical features

We were able to obtain data from 373 patients with liver cancer included within the TCGA, and we obtained key information

Table 2

The association between BDH1 mRNA expression and clinical parameters in liver cancer patients.

Clinical characteristics	Variable	No. of patients	BDH1 mRNA expression		$\chi 2$	*P*-value
			High (%)	Low (%)		
Age	<55	117	39 (26.53)	78 (34.67)	2.3656	.1103
	>=55	255	108 (73.47)	147 (65.33)		
Gender	FEMALE	121	37 (25.17)	84 (37.17)	5.3156	**.0175**
	MALE	252	110 (74.83)	142 (62.83)		
Histological type	Fibrolamellar Carcinoma	3	0 (0)	3 (1.33)	3.9016	.1686
	Hepatocellular Carcinoma	363	146 (99.32)	217 (96.02)		
	Hepatocholangiocarcinoma (Mixed)	7	1 (0.68)	6 (2.65)		
Histologic grade	G1	55	29 (20.14)	26 (11.61)	19.8989	**.0001**
	G2	178	81 (56.25)	97 (43.3)		
	G3	123	33 (22.92)	90 (40.18)		
	G4	12	1 (0.69)	11 (4.91)		
Stage	I	172	91 (67.41)	81 (37.85)	32.4804	**.000**
	II	87	27 (20)	60 (28.04)		
	III	85	17 (12.59)	68 (31.78)		
	IV	5	0 (0)	5 (2.34)		
T classification	T1	182	95 (65.52)	87 (38.5)	31.5745	**.000**
	T2	95	30 (20.69)	65 (28.76)		
	T3	80	18 (12.41)	62 (27.43)		
	T4	13	1 (0.69)	12 (5.31)		
	TX	1	1 (0.69)	0 (0)		
N classification	N0	253	96 (65.31)	157 (69.78)	3.998	.1334
	N1	4	0 (0)	4 (1.78)		
	NX	115	51 (34.69)	64 (28.44)		
M classification	M0	267	98 (66.67)	169 (74.78)	6.6012	**.0352**
	M1	4	0 (0)	4 (1.77)		
	MX	102	49 (33.33)	53 (23.45)		
Radiation therapy	NO	340	137 (99.28)	203 (96.67)	1.4954	.1531
	YES	8	1 (0.72)	7 (3.33)		
Residual tumor	R0	326	135 (93.1)	191 (86.43)	5.0842	.1395
	R1	17	3 (2.07)	14 (6.33)		
	R2	1	0 (0)	1 (0.45)		
	RX	22	7 (4.83)	15 (6.79)		
Vital status	DECEASED	130	36 (24.49)	94 (41.59)	10.7337	**.0008**
	LIVING	243	111 (75.51)	132 (58.41)		
Sample_type	Primary Tumor	371	146 (99.32)	225 (99.56)	0	1
	Recurrent Tumor	2	1 (0.68)	1 (0.44)		
Overall survival	NO	237	111 (75.51)	126 (57.27)	12.0279	**.0004**
	YES	130	36 (24.49)	94 (42.73)		
Relapse-free survival	NO	179	84 (66.14)	95 (49.22)	8.2226	**.0039**
	YES	141	43 (33.86)	98 (50.78)		

P -value in bold represent significant clinical significance ($P \leq .05$).

regarding these patients including age, gender, tumor histological grade, TNM stage and survival outcomes. We further analyzed BDH1 mRNA expression in these samples based upon RNA-seq results (Table 1). Patient pTNM and cTNM stages were determined according to AJCC standards (8th edition).[21]

3.2. Liver cancer samples exhibit low BDH1 mRNA expression

We found that BDH1 mRNA expression was markedly decreased in liver cancer samples relative to normal control tissue samples ($P = 3.3e^{-16}$, Fig. 1). We further found that surviving patients has significantly higher BDH1 mRNA expression at the time of analysis than did patients who were deceased ($P = .0016$). In addition, BDH1 mRNA expression tended to decrease as clinical ($P = 1.1e^{-08}$), histological ($P = 1.3e^{-05}$), or T stage increased ($P = 3.8e^{-08}$, Fig. 1). We also found that BDH1 mRNA expression was significantly linked with patient age ($P = .0072$) and gender ($P = .021$, Fig. 1).

3.3. Assessment of the relationship between BDH1 mRNA expression and clinical parameters

We next assessed the clinical relevance of BDH1 mRNA expression levels in patients with liver cancer by comparing this expression to relevant clinical parameters (Table 2). We found that BDH1 mRNA expression was significantly correlated with gender ($P = .0175$), histologic grade ($P = .0001$), stage ($P < .001$), T classification ($P < .001$), M classification ($P = .0352$), vital status ($P = .0008$), overall survival ($P = .0004$), and relapse-free survival ($P = .0039$). Levels of BDH1 mRNA expression tended to reduce with increases in clinical stage, histologic grade, or T classification (Table 2).

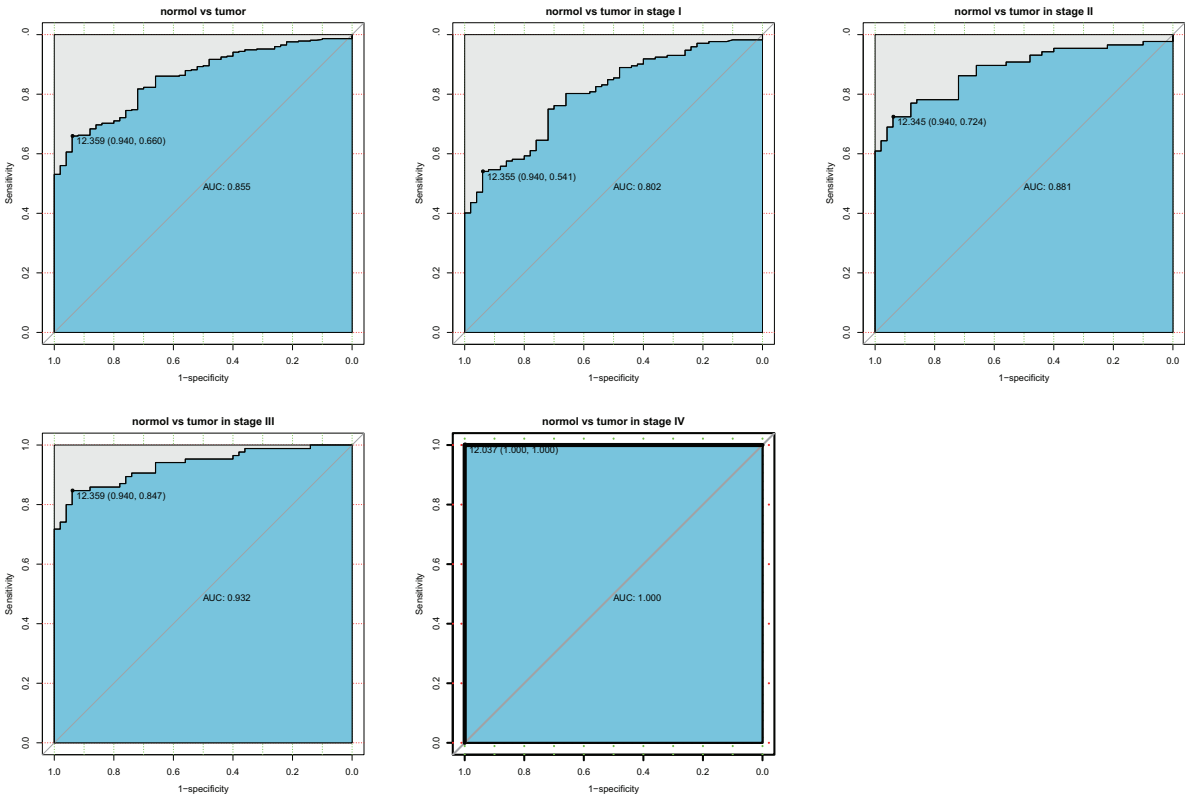

Figure 2. Assessment of the diagnostic value of BDH1 mRNA in liver cancer based on a ROC curve. AUC = area under the curve, ROC = receiver-operating characteristic.

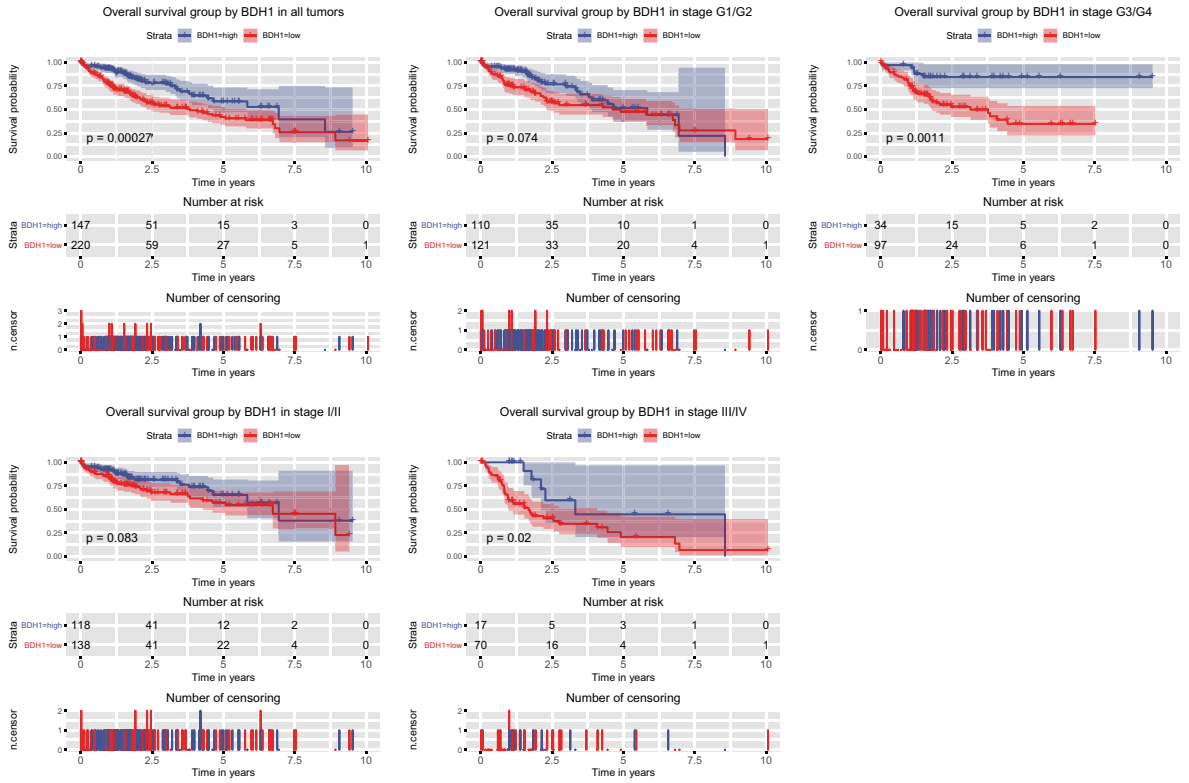

Figure 3. Assessment of differences in patient overall survival as a function of BDH1 mRNA expression and different clinical parameters. Clinical parameters assessed included clinical stage (I/II, III / IV) and histologic grade (G1/G2, G3/G4).

Table 3

The association between BDH1 mRNA expression, other clinical parameters, and liver cancer patient overall survival.

	Univariate analysis			Multivariate analysis		
Parameters	**Hazard ratio**	**95%CI (lower~upper)**	***P* value**	**Hazard ratio**	**95%CI (lower-upper)**	***P* value**
Age	1.00	0.69–1.45	.997			
Gender	0.80	0.56–1.14	.220			
Histological type	0.99	0.27–3.66	.986			
Histologic grade	1.04	0.84–1.3	.698			
Stage	1.38	1.15–1.66	.001	0.86	0.7–1.07	.183
T classification	1.66	1.39–1.99	.000	1.74	1.38–2.19	.000
N classification	0.73	0.51–1.05	.086			
M classification	0.72	0.49–1.04	.077			
Radiation therapy	0.51	0.26–1.03	.060			
Residual Tumor	1.42	1.13–1.8	.003	1.39	1.08–1.78	.009
BDH1	2.02	1.37–2.96	.000	1.60	1.08–2.38	.020

P -value in bold represent significant clinical significance ($P \leq .05$).

3.4. BDH1 mRNA levels as a diagnostic biomarker in liver cancer

Our results suggest that BDH1 may serve as an optimal diagnostic biomarker for liver cancer based upon an AUC analysis (AUC=0.855, Fig. 2). We further found that as the clinical stage of cancer increased from stage I to IV, the diagnostic potential of BDH1 correspondingly rose to a maximum of 100% at stage IV (AUC: 0.802 for stage I, 0.881 for stage II, 0.932 for stage III, 1.000 for stage IV, Fig. 2).

3.5. BDH1 mRNA as a prognostic biomarker in liver cancer

We found that on average liver cancer patients expressing high BDH1 levels had a longer overall survival as compared to those

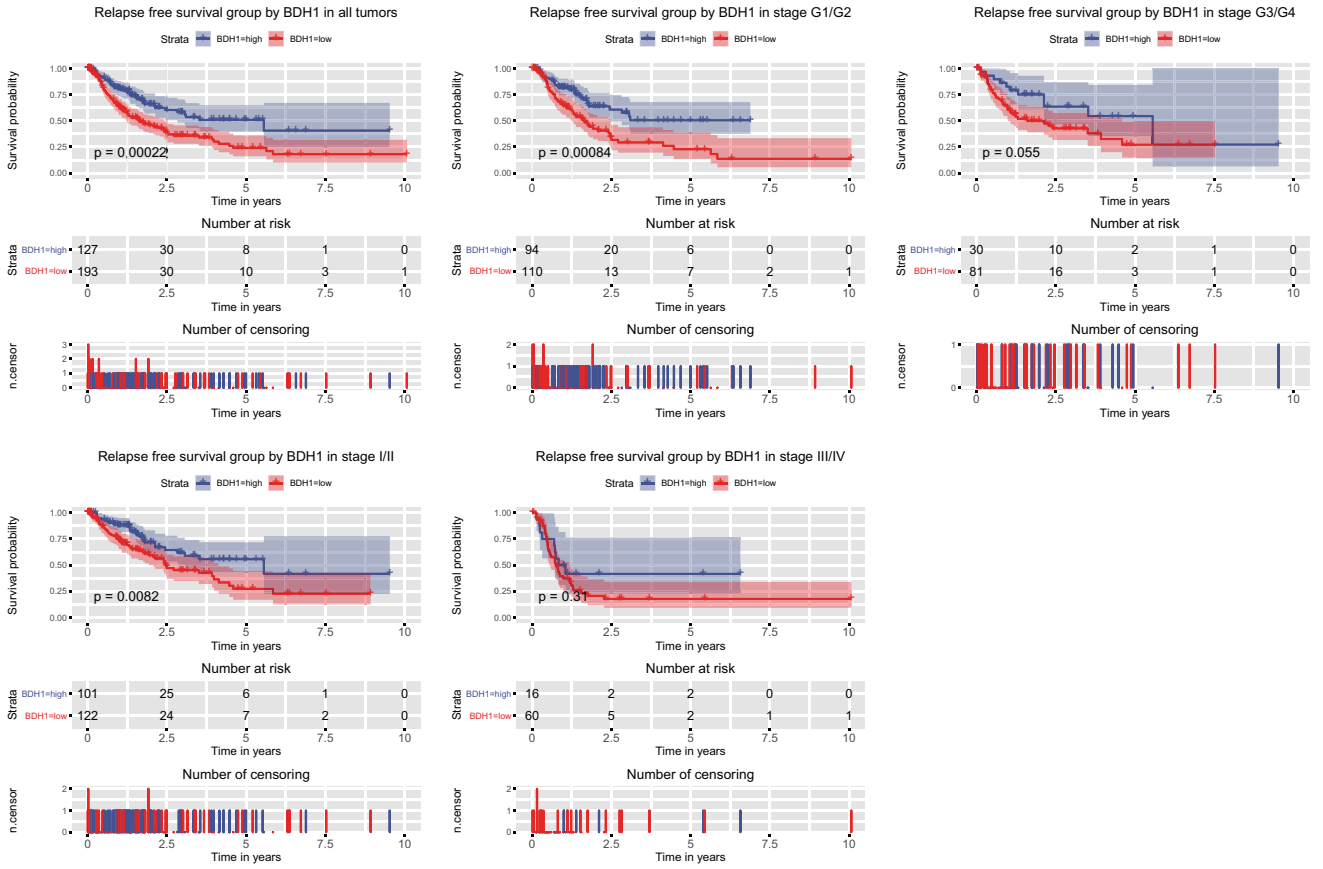

Figure 4. Assessment of differences in patient relapse-free survival as a function of BDH1 mRNA expression and different clinical parameters. Clinical parameters assessed included clinical stage (I/II, III / IV) and histologic grade (G1/G2, G3/G4).

Table 4

The association of BDH1 mRNA expression, other clinical parameters, and liver cancer patient relapse-free survival.

	Univariate analysis			Multivariate analysis		
Parameters	**Hazard Ratio**	**95%CI (lower~upper)**	***P* value**	**Hazard Ratio**	**95%CI (lower-upper)**	***P* value**
Age	0.90	0.63–1.28	.550			
Gender	0.99	0.7–1.41	.966			
Histological type	2.02	0.66–6.24	.220			
Histologic grade	0.98	0.8–1.21	.883			
Stage	1.66	1.38–1.99	.000	1.11	0.87–1.43	.4
T classification	1.78	1.49–2.12	.000	1.58	1.21–2.05	.001
N Classification	0.97	0.67–1.4	.874			
M classification	1.17	0.79–1.74	.432			
Radiation therapy	0.74	0.26–2.16	.584			
Residual tumor	1.28	1.01–1.61	.042	1.33	1.05–1.68	.02
BDH1	1.94	1.36–2.78	.003	1.55	1.07–2.24	.021

P -value in bold represent significant clinical significance ($P \leq .05$).

expressing low levels of BDH1 ($P = .00027$, Fig. 3). When we examined patient subsets, we similarly detected lower overall survival in patients with low BDH1 expression who were classified as G3/G4 ($P = .0011$) or stage III/IV ($P = .02$, Fig. 3). We then used a univariate analysis as a means of identifying relevant variables that were altered between patient groups, with identified variables including clinical stage, T stage, residual tumor status, and BDH1 expression. We then conducted a

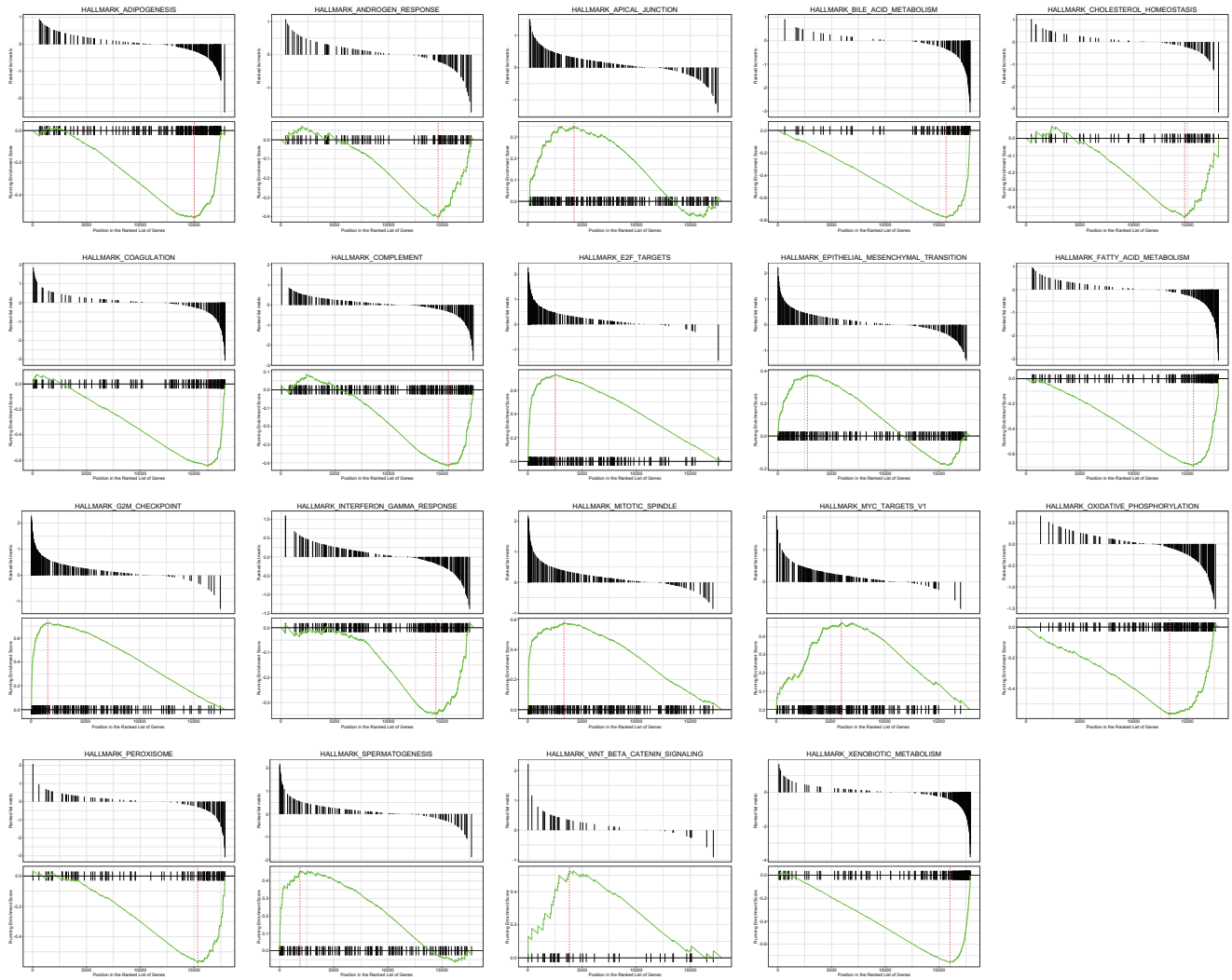

Figure 5. Assessment of BDH1-related signaling pathways.

Table 5

The Gene sets significantly associated with expression levels of BDH1.

Gene set name	NES	NOM *P*-value	FDR q-value
HALLMARK_SPERMATOGENESIS	1.72559	.002004	0.004262
HALLMARK_E2F_TARGETS	2.909997	.002033	0.004262
HALLMARK_G2M_CHECKPOINT	2.88629	.002033	0.004262
HALLMARK_MYC_TARGETS_V1	1.88039	.002058	0.004262
HALLMARK_MITOTIC_SPINDLE	2.299837	.002105	0.004262
HALLMARK_APICAL_JUNCTION	1.387843	.004149	0.007402
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	1.502239	.004219	0.007402
HALLMARK_WNT_BETA_CATENIN_SIGNALING	1.605654	.011605	0.017965
HALLMARK_COMPLEMENT	−1.61651	.001908	0.004262
HALLMARK_XENOBIOTIC_METABOLISM	−2.94982	.001916	0.004262
HALLMARK_ADIPOGENESIS	−2.09013	.001931	0.004262
HALLMARK_OXIDATIVE_PHOSPHORYLATION	−2.18661	.001961	0.004262
HALLMARK_FATTY_ACID_METABOLISM	−2.59892	.00198	0.004262
HALLMARK_COAGULATION	−2.38431	.001988	0.004262
HALLMARK_BILE_ACID_METABOLISM	−2.75961	.002033	0.004262
HALLMARK_PEROXISOME	−1.99336	.002053	0.004262
HALLMARK_INTERFERON_GAMMA_RESPONSE	−1.34707	.009488	0.015605
HALLMARK_ANDROGEN_RESPONSE	−1.41688	.01417	0.019867
HALLMARK_CHOLESTEROL_HOMEOSTASIS	−1.53702	.014344	0.019867

Gene sets with combine NOM *P* value <.05 and FDR<0.25 were regarded as significant.
FDR = false discovery rate, NES = normalized enrichment score, NOM = nominal.

multivariate analysis that indicated that low BDH1 expression was an independent prognostic indicator in liver cancer patients ($P = .020$), as was T stage ($P < .001$) and residual tumor status ($P = .009$, Table 3).

We further extended these analyses to examine relapse-free survival in these same patients, revealing patients with high BDH1 to have longer average relapse-free survival ($P = .00022$, Fig. 4). A subset examination revealed that patients with high BDH1 expression, in addition to being classified as G1/G2 ($P = .00084$) and stage I/ II ($P = .0082$, Fig. 4), had a favorable prognoses and better relapse-free survival. We additionally determined that BDH1 ($P = .021$), T stage ($P = .001$), and residual tumor ($P = .02$) were all independent prognostic indicators of relapse-free survival (Table 4).

3.6. Assessment of BDH1-related signaling pathways

We next aimed to identify the signaling pathways which may be influenced by BDH1 expression in liver cancer. To identify these genes, we conducted a GSEA assessment in which defined gene sets that are significantly enriched in a given sample subset are identified. By comparing BDH1 high and low liver cancer patient datasets with $P < .05$ and false discovery rate < 0.25 as thresholds, we found that signaling pathways linked to spermatogenesis, epithelial mesenchymal transition (EMT), the apical junction, the mitotic spindle, E2F targets, the G2M checkpoint, MYC, and wnt/β-catenin signaling were all highly enriched in BDH1-high samples (Fig. 5, Table 5). In contrast, pathways linked to adipogenesis, oxidative phosphorylation, and fatty acid metabolism were enriched in BDH1-low samples (Table 5).

4. Discussion

There are many ongoing efforts aimed at improving the prognosis of liver cancer, in part via identifying novel and specific prognostic markers of patient outcomes. As such, we have been focused on identifying biomarkers significantly correlated with patient prognosis.[22–29] In the present study, we determined that BDH1 mRNA is expressed at lower levels in liver cancer, and that this low BDH1 mRNA expression is an independent predictor of poor liver cancer patient outcomes. BDH1 mRNA expression levels were significantly associated with histologic grade, clinical stage, T and M classifications, and survival.

It is not surprising that BDH1 mRNA was found to be downregulated in human liver cancer. The expression level of BDH1 mRNA was found to be significantly decreased in rat liver cancer tissues and rat liver cancer cell lines.[13,14] In addition, compared with well-differentiated rat liver cancer samples, poorly differentiated liver cancer tissues have significantly lower BDH1 mRNA expression and activity.[13] These results are consistent with our findings in human liver cancer. However, Huang et al[30] observed no significant differences when comparing the expression of BDH1 mRNA in cancer and non-cancerous tissues. The inconsistency between these results and those of our study may be due to their small number of cancerous tissues (n = 20). In addition, their samples selection was limited to a single site and may not have been representative. Moreover, we also found that the changes of BDH1 mRNA expression in liver cancer were moderately associated with liver cancer diagnosis, such that as liver cancer clinical stage increased, so too did the diagnostic specificity and sensitivity of BDH1 as a diagnostic biomarker, with an AUC as high as 1.000 for stage IV disease.

The mechanism of BDH1 mRNA downregulation in liver cancer tissues remains unclear. Weinhouse and Churchill et al[13,14] have shown that a decrease in the level of functional BDH1 mRNA expression in rat liver cancer cells leads to a decreased BDH1 activity in mitochondria, accompanied by an increase in CoA transferase activity. This may enable tumor cells to produce more energy to meet their higher energy needs, thereby supporting their increased rates of growth and protein synthesis. This may explain why those liver cancer patients with

lower BDH1 mRNA expression exhibited worse disease outcomes with respect to differentiation, clinical, T stages and poorer survival. However, recent studies have shown that BDH1 upregulation can also promote tumor development. Michael et al[31] upregulated BDH1 expression in cells via transfection, demonstrating that this led to enhanced ketone metabolism, thereby driving the anabolic growth and metastasis of human breast cancer cells. In addition, Huang et al[30] found that BDH1 mRNA expression and BDH1 activity were significantly increased in nutrient-starved human liver cancer cells, thereby reactivating ketolysis to promote cell proliferation. These may be the result of cancer cells adapting to metabolic challenges and opening themselves to utilizing a wide range of nutrient sources under nutritional constraints.[32–34] At the same time, combined with our GSEA results show that in the environment of high expression of BDH1, the activated signal pathways of oncogenes, such as MYC[35] and wnt/β-catenin[36] signaling, are highly enriched.

We found that BDH1 mRNA expression was significantly correlated with liver cancer prognosis. To date, a few previous studies have assessed BDH1 expression in certain human cancer types. For example, Diamandis et al[37] found that BDH1 expression was elevated in high-grade prostate cancer tissues. A study of breast cancer identified a higher rate of unique non-synonymous BDH1 RNA variants in tumors relative to healthy tissue samples.[38] Our results suggest that BDH1 mRNA can also serve as a prognostic biomarker for liver cancer, as patients with lower BDH1 mRNA expression have poorer survival and outcomes, especially in those with stage I/II and histologic grade G1/G2 disease. As such, assessment of BDH1 mRNA expression may offer valuable potential as a means of conducting personalized precision medicine in liver cancer patients.

5. Conclusion

As far as we are aware, this study is the first to date exploring the link between BDH1 mRNA expression, clinical parameters, and outcomes in liver cancer patients. Based on our findings, BDH1 mRNA appears to be an independent prognostic marker that can predict survival in patients. To expand on these findings, in the future we will explore the mechanistic role of BDH1 protein in liver cancer, and we will collect additional liver cancer samples to improve our prognostic BDH1 mRNA expression-based model.

Author contributions

Conceptualization: Yan Jiao, Yunpeng Liu.
Data curation: Yan Jiao.
Formal analysis: Yanqing Li.
Investigation: Yan Jiao.
Methodology: Yanqing Li, Yan Jiao.
Project administration: Ying Liu.
Resources: Ying Liu.
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