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Original Article

NCEH1 may be a prognostic biomarker for pancreatic cancer

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Abstract: Neutral Cholesterol Ester Hydrolase 1 (NCEH1) is an enzyme involved in ether lipid metabolism, and the *NCEH1* gene is overexpressed in a variety of tumors. However, its role in pancreatic cancer remains unknown. Therefore, we compared the gene transcription data of healthy and pancreatic cancer tissues using the Cancer Genome Atlas and Genotype-Tissue Expression databases. R software (v3.6.1) was used for the differential, clinicopathological correlation, and survival analyses. We found that *NCEH1* was overexpressed in pancreatic cancer tissues compared with that in healthy tissues (P = 1.732 e-50), and that its expression level was related to lymph node metastasis. High *NCEH1* expression was associated with poor overall survival (P = 0.002). Using univariate and multivariate Cox regression analyses, we determined that *NCEH1* is an independent risk factor for pancreatic cancer. Gene set enrichment analysis identified that *NCEH1* overexpression is prominent in cell-cell adhesion junctions, pancreatic cancer, cancer-associated pathways, prostate cancer, and chronic myeloid leukemia. In contrast, low *NCEH1* expression correlated to high oxidative phosphorylation. Thus, we conclude that *NCEH1* may be a prognostic biomarker for pancreatic cancer.

Keywords: NCEH1, prognostic biomarker, pancreatic cancer, TCGA, bioinformatics analysis, leading edge analysis

Introduction

Pancreatic cancer (PC) is difficult to diagnose, lacks an effective treatment, and was the seventh leading cause of cancer deaths in 2018 [1]. Despite the recent advancements in surgical treatment and targeted drug therapy, PC still has poor response to drugs owing to its high heterogeneity [2]. For treatment to be effective, it is necessary to discover novel prognostic biomarkers to correctly identify the type of PC [3].

The neutral cholesterol ester hydrolase 1 (NCEH1) (also known as KIAA1363 or AADACL1) is enzyme that hydrolyzes 2-acetyl monoalkylglycerol in the metabolism of ether lipids in cancer cells [4]. It is encoded by the *NCEH1* gene located on chromosome 3. Previously, the overexpression of *NCEH1* in a variety of tumors, such as ovarian [5] and breast cancers [6], has been observed. Moreover, a study has found that inhibiting the activity of *NCEH1* can prevent the migration and growth of cancer cells [7]. Therefore, we hypothesized that *NCEH1* can be a target for cancer treatment.

In this study, using expression profile data of pancreatic cancer from public databases, we analyzed the relationship among the transcriptional level of *NCEH1*, its clinicopathologic characteristics, and prognosis of patients with pancreatic cancer. We further explored the functional pathways of *NCEH1* using gene set enrichment analysis (GSEA) [8].

Materials and methods

Data mining

All datasets were downloaded from the UCSC Xena browser (http://xena.ucsc.edu/), including the PC transcriptome and clinical data from The Cancer Genome Atlas (TCGA) [9] and the transcriptome data of healthy pancreatic cells from the Genotype-Tissue Expression (GTEx) database [10]. The data were merged and corrected by the "BiocManager" package in the R software (v3.6.1) [11].

Table 1. Clinical characteristics of 175 pancreatic cancer patients

Characteristic	Number of patients (%)
(1) Age	
≤ 65	90 (51.4)
> 65	85 (48.6)
(2) Gender	
Male	97 (55.4)
Female	78 (44.6)
(3) Histologic grade	
G1	29 (16.6)
G2	93 (53.1)
G3	51 (29.1)
G4	2 (1.1)
(4) Clinical stage	
Stage I	18 (10.3)
Stage II	148 (84.6)
Stage III	4 (2.3)
Stage IV	5 (2.9)
(5) T classification	
T1	5 (2.9)
T2	23 (13.1)
T3	143 (81.7)
T4	4 (2.3)
(6) N classification	
N0	48 (27.4)
N1	127 (72.6)
(7) Survival status	
Survived	83 (47.4)
Dead	92 (52.6)

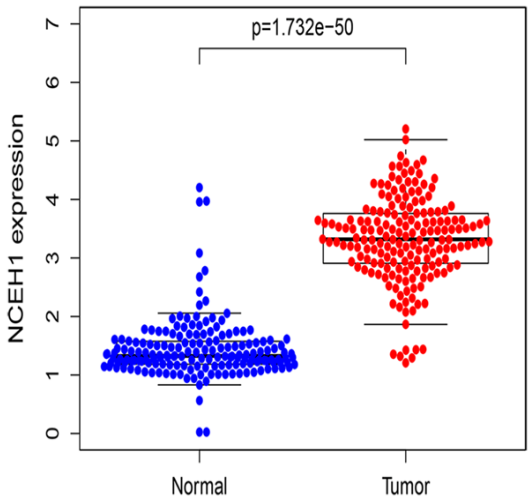

Figure 1. *NCEH1* expression levels in healthy and pancreatic tumor tissues.

Statistical analysis

R software was used for the analyses. The difference in the *NCEH1* expression levels between healthy and tumor pancreatic tissues is represented by a scatter plot using the "beeswarm" package. The Wilcoxon test [12] was used to assess the association between NCEH1 and the clinicopathologic features of pancreatic cancer. Samples with unknown clinicopathologic status were excluded from the analysis. The correlation with survival was calculated using the "survival.R" package. Univariate and multivariate Cox regression analyses were used to assess whether NCEH1 can be used as an independent predictor of survival. Results were considered significant when $P < 0.05$.

Gene set enrichment analysis

JAVA [13] was used for GSEA. A leading-edge subset in the enrichment plot was suspected to be a pathway involving *NCEH1* in the development of pancreatic cancer. The gene sets were considered significant when nominal $P < 0.050$ and false discovery rate $q < 0.250$.

Results

Data characteristics

A total of 349 transcripts were downloaded from the UCSC Xena browser (TCGA, $n = 182$; GTEx, $n = 167$). Of which, 178 were from pancreatic cancer patients and 171 were from healthy individuals. After omitting those with incomplete clinical information, 175 pancreatic cancer cases were included in this study (**Table 1**). In the TNM (tumor-node-metastasis) classification, the M classification was not considered because a significant amount of data was missing.

NECH1 expression levels in PC patients

From the differential analysis, the expression level of *NCEH1* in cancer tissues was significantly higher than that in healthy tissues ($P = 1.732$ e-50) (**Figure 1**). Wilcoxon test indicated that the expression level of *NCEH1* is related to the N classification in patients with pancreatic cancer ($P = 0.039$). Further, *NCEH1* expression was higher in patients with regional lymph node involvement than in the control group (**Figure 2**).

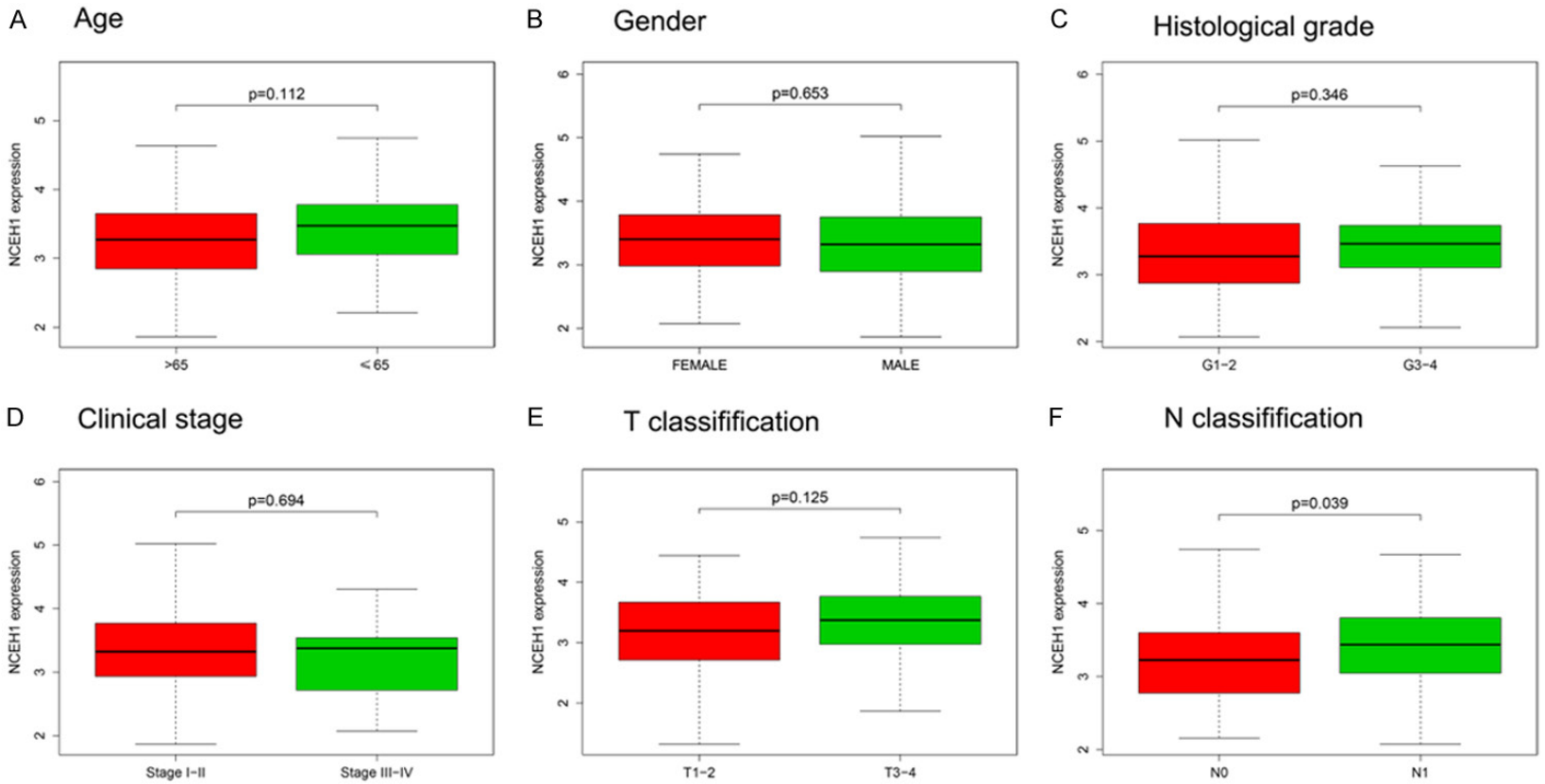

Figure 2. Relationships between *NCEH1* expression and the clinicopathologic characteristics of pancreatic cancer: (A) age, (B) gender, (C) histologic grade, (D) clinical stage, (E) T classification, and (F) N classification.

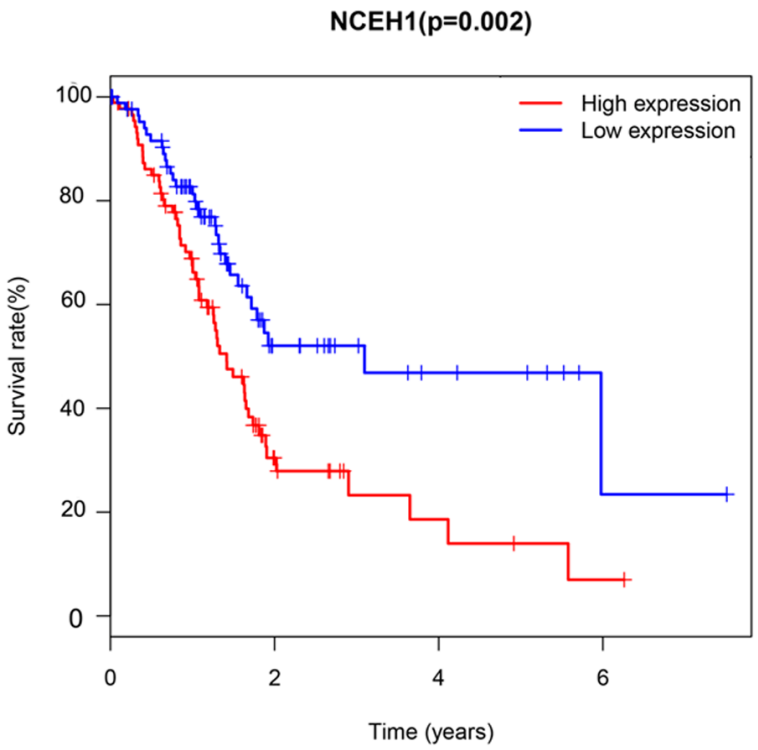

Figure 3. The Kaplan-Meier curve shows the relationship between *NCEH1* expression levels and overall survival rate.

Table 2. Univariate and multivariate Cox regression analyses of the correlation between *NCEH1* expression levels and OS in patients with pancreatic cancer

Variable	Univariate analysis		Multivariate analysis	
	HR [95% CI]	*P* value	HR [95% CI]	*P* value
(1) Age	1.03 [1.01-1.05]	0.016	1.03 [1.00-1.05]	0.021
(2) Gender	0.78 [0.51-1.20]	0.259		
(3) Histologic grade	1.39 [0.88-2.17]	0.155		
(4) Clinical stage	0.89 [0.28-2.82]	0.841		
(5) T classification	2.03 [1.01-4.08]	0.046	1.29 [0.62-2.66]	0.500
(6) N classification	2.26 [1.31-3.90]	0.003	1.69 [0.93-3.09]	0.080
(7) NCEH1	1.69 [1.24-2.29]	< 0.001	1.58 [1.14-2.18]	0.006

Table 3. Signaling pathways associated with *NCEH1* high- and low-expression phenotypes

Pathways	Nominal Enrichment Score	Nominal *p*-value	False Discovery Rate *q*-value
KEGG_ADHERENS_JUNCTION	2.23	< 0.001	0.005
KEGG_PANCREATIC_CANCER	2.22	< 0.001	0.003
KEGG_PATHWAYS_IN_CANCER	2.19	< 0.001	0.004
KEGG_PROSTATE_CANCER	2.15	< 0.001	0.004
KEGG_CHRONIC_MYELOID_LEUKEMIA	2.15	< 0.001	0.003
KEGG_OXIDATIVE_PHOSPHORYLATION	-2.00	0.008	0.046

Prognostic value of NCEH1 in pancreatic cancer

The Kaplan-Meier curve showed that high a *NCEH1* expression is associated with poor survival rates (P = 0.002; **Figure 3**). After a univariate Cox analysis, variables with P < 0.050 were included in the multivariate Cox regression analysis. The results showed that a high *NCEH1* expression is an independent risk factor for poor prognosis in patients with pancreatic cancer (hazard ratio [HR] = 1.58; 95% confidence interval [CI] = 1.14-2.18; P = 0.006) (**Table 2**).

NCEH1 expression-related signaling pathways

From the GSEA, we obtained multiple signaling pathways that are significantly enriched (Supplementary Table 1) for both high and low *NCEH1* expression levels. **Table 3** and **Figure 4** show the significantly enriched gene sets, ordered according to their Normalized Enrichment Score (NES) values. We identified the top five signaling pathways significantly enriched for the *NCEH1* high-expression phenotype: "KEGG_ADHERENS_JUNCTION", "KEGG_PANCREATIC_CANCER", "KEGG_PATHWAYS_IN_CANCER", "KEGG_PROSTATE_CANCER", and "KEGG_CHRONIC_MYELOID_LEUKEMIA". The "KEGG_OXIDATIVE_PHOSPHORYLATION" pathway was associated with significantly low expression of *NCEH1*.

Discussion

The survival rate for patients with localized lesions in the pancreas is high [14] because surgery is the only available treatment for pancreatic can-

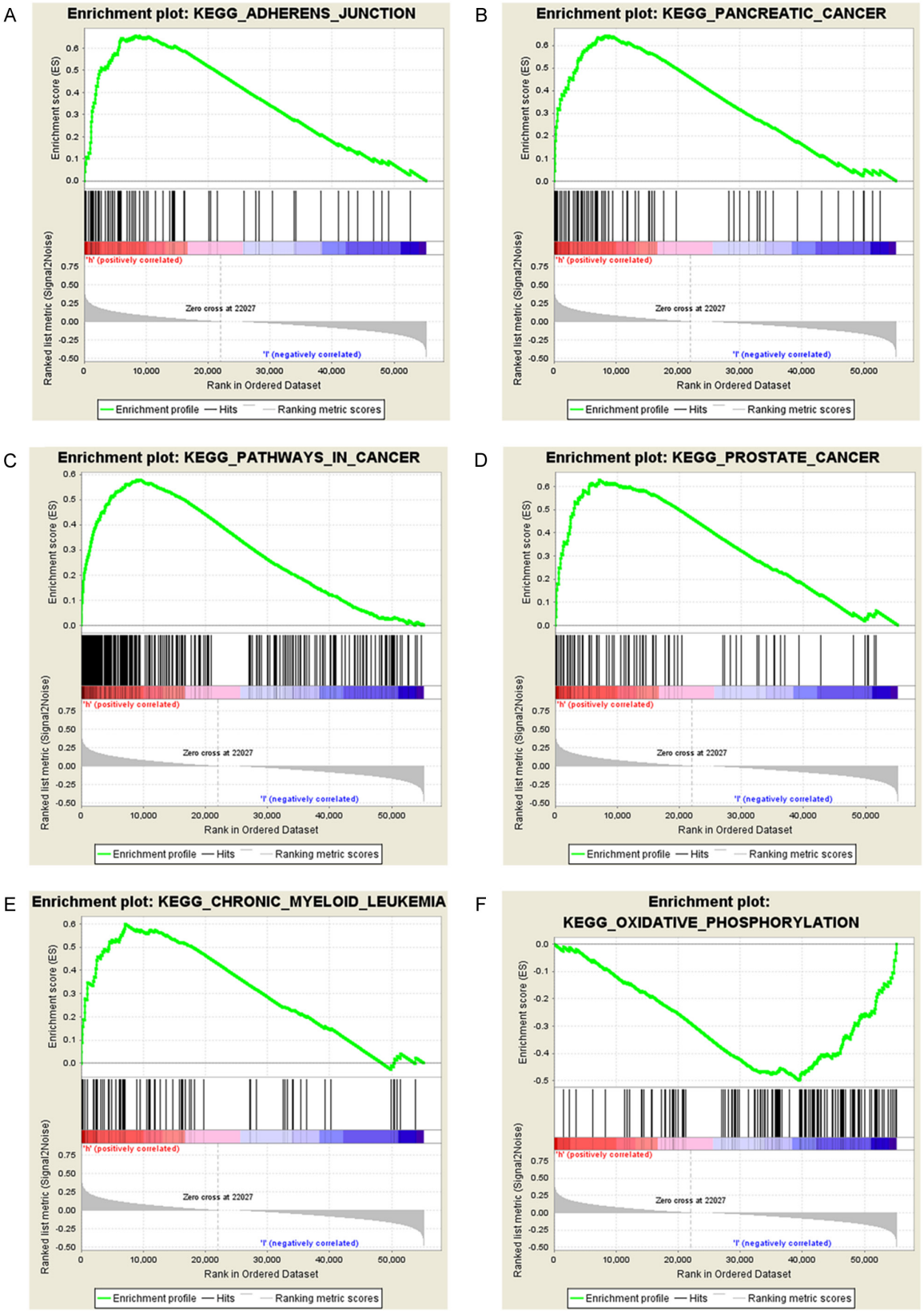

Figure 4. Enrichment plots of the *NCEH1* high- and low-expression phenotype using GSEA. (A) KEGG_ADHERENS_JUNCTION, (B) KEGG_PANCREATIC_CANCER, (C) KEGG_PATHWAYS_IN_CANCER, (D) KEGG_PROSTATE_CANCER, (E) KEGG_CHRONIC_MYELOID_LEUKEMIA, and (F) KEGG_OXIDATIVE_PHOSPHORYLATION.

cer [15]. Tumor metastasis decreases the chance of treatment by surgery. The *NCEH1* gene codes for a serine hydrolase, which is positively correlated with tumor invasion through activity-based protein profiling (ABPP) [16]. However, many studies have contradicted the aforementioned results as the *NCEH1* gene has been indicated to promote tumor progression by inhibiting enzyme activity [16, 17]. Our results were consistent with these studies and showed that higher *NCEH1* transcription levels were observed in patients with lymph node-metastatic pancreatic cancer.

Elevated ether lipid metabolism is one of the characteristics of cancer cells [18]. *NCEH1* is involved in ether lipid metabolism and is robustly expressed in macrophages [19]. Previously, through mRNA extraction and Affymetrix GeneChip Hybridization, Iacobuzio-Donahue et al. [20] found a significant difference in *NCEH1* expression levels between normal and pancreatic cancer tissues, consistent with our findings. We also found that the expression level of *NCEH1* in patients with pancreatic cancer is related to the N classification ($P = 0.039$), indicating that patients with local lymph node involvement express higher levels of *NCEH1*.

To the best of our knowledge, this is the first study to demonstrate the correlation between *NCEH1* expression levels and tumor survival prognosis. We have shown that *NCEH1* can be used as an independent prognostic biomarker in patients with pancreatic cancer, and that high level of *NCEH1* is a predictor of poor prognosis. Furthermore, using GSEA, we found that the *NCEH1*-overexpression phenotype is enriched in cell-cell adhesion junctions [21], which suggests that *NCEH1* may be involved in cancer progression by affecting cell-cell adhesion, cell migration, and signaling. *NCEH1* is also enriched in pancreatic cancer, prostate cancer, chronic myeloid leukemia, and other signaling pathways related to tumor progression, thus indicating a functional role for *NCEH1*.

This study used the transcriptome data from internationally recognized and continuously updated TCGA databases for prognostic analysis. Further, the differential analysis we performed included the normal genome data of GTEx, which was used to adjust the imbalance between the two groups, thus improving the accuracy and reliability of our results However, there exists no clinical study that can verify our findings. Thus, large-scale clinical trials investigating the potential of *NCEH1* as a prognostic gene for PC are warranted.

In conclusion, our study demonstrated the potential of *NCEH1* as a prognostic biomarker for pancreatic cancer. We have shown a correlation between *NCEH1* expression levels and the occurrence of lymph node metastasis, as well as that between higher *NCEH1* expression and poor prognosis.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Multiple signaling pathways that are significantly enriched for NCEH1 expression levels

	Pathways	Nominal Enrichment Score	Nominal *p*-value	False Discovery Rate *q*-value
1	KEGG_ADHERENS_JUNCTION	2.23	0.000	0.005
2	KEGG_PANCREATIC_CANCER	2.22	0.000	0.003
3	KEGG_PATHWAYS_IN_CANCER	2.19	0.000	0.004
4	KEGG_PROSTATE_CANCER	2.15	0.000	0.004
5	KEGG_CHRONIC_MYELOID_LEUKEMIA	2.15	0.000	0.003
6	KEGG_NON_SMALL_CELL_LUNG_CANCER	2.12	0.000	0.003
7	KEGG_SMALL_CELL_LUNG_CANCER	2.11	0.000	0.003
8	KEGG_RENAL_CELL_CARCINOMA	2.06	0.000	0.004
9	KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	2.05	0.000	0.004
10	KEGG_TGF_BETA_SIGNALING_PATHWAY	2.05	0.000	0.004
11	KEGG_ENDOCYTOSIS	2.03	0.000	0.005
12	KEGG_MELANOMA	2.01	0.000	0.006
13	KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	2.01	0.002	0.006
14	KEGG_ENDOMETRIAL_CANCER	2.00	0.000	0.006
15	KEGG_GLIOMA	1.99	0.000	0.007
16	KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION	1.99	0.000	0.006
17	KEGG_COLORECTAL_CANCER	1.98	0.000	0.006
18	KEGG_ACUTE_MYELOID_LEUKEMIA	1.97	0.000	0.007
19	KEGG_DORSO_VENTRAL_AXIS_FORMATION	1.96	0.002	0.007
20	KEGG_AXON_GUIDANCE	1.96	0.000	0.007
21	KEGG_FOCAL_ADHESION	1.96	0.000	0.007
22	KEGG_APOPTOSIS	1.96	0.004	0.007
23	KEGG_ERBB_SIGNALING_PATHWAY	1.95	0.000	0.007
24	KEGG_THYROID_CANCER	1.94	0.002	0.007
25	KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.91	0.002	0.009
26	KEGG_ECM_RECEPTOR_INTERACTION	1.91	0.000	0.009
27	KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	1.89	0.000	0.012
28	KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	1.87	0.004	0.014
29	KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	1.85	0.008	0.016
30	KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.85	0.002	0.016
31	KEGG_TIGHT_JUNCTION	1.84	0.002	0.017
32	KEGG_LYSOSOME	1.84	0.019	0.017
33	KEGG_BLADDER_CANCER	1.81	0.006	0.022

34	KEGG_CELL_ADHESION_MOLECULES_CAMS	1.80	0.008	0.023
35	KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	1.80	0.006	0.022
36	KEGG_LEISHMANIA_INFECTION	1.77	0.017	0.029
37	KEGG_JAK_STAT_SIGNALING_PATHWAY	1.77	0.012	0.029
38	KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	1.76	0.019	0.031
39	KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	1.74	0.026	0.038
40	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	1.73	0.026	0.039
41	KEGG_HEDGEHOG_SIGNALING_PATHWAY	1.72	0.010	0.042
42	KEGG_SPHINGOLIPID_METABOLISM	1.71	0.015	0.047
43	KEGG_GAP_JUNCTION	1.70	0.004	0.049
44	KEGG_OOCYTE_MEIOSIS	1.69	0.012	0.05
45	KEGG_WNT_SIGNALING_PATHWAY	1.69	0.004	0.049
46	KEGG_N_GLYCAN_BIOSYNTHESIS	1.68	0.030	0.055
47	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	1.67	0.018	0.055
48	KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	1.66	0.050	0.059
49	KEGG_P53_SIGNALING_PATHWAY	1.66	0.017	0.06
50	KEGG_CHEMOKINE_SIGNALING_PATHWAY	1.64	0.043	0.067
51	KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.64	0.027	0.068
52	KEGG_MAPK_SIGNALING_PATHWAY	1.64	0.004	0.066
53	KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	1.62	0.010	0.071
54	KEGG_BASAL_CELL_CARCINOMA	1.61	0.035	0.075
55	KEGG_GRAFT_VERSUS_HOST_DISEASE	1.61	0.043	0.075
56	KEGG_MTOR_SIGNALING_PATHWAY	1.59	0.025	0.082
57	KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	1.58	0.014	0.083
58	KEGG_INSULIN_SIGNALING_PATHWAY	1.58	0.034	0.083
59	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	1.57	0.046	0.09
60	KEGG_INOSITOL_PHOSPHATE_METABOLISM	1.56	0.035	0.093
61	KEGG_VIBRIO_CHOLERAE_INFECTION	1.56	0.018	0.092
62	KEGG_MELANOGENESIS	1.52	0.029	0.113
63	KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_LACTO_AND_NEOLACTO_SERIES	1.51	0.046	0.113
64	KEGG_VEGF_SIGNALING_PATHWAY	1.50	0.042	0.119
65	KEGG_ETHER_LIPID_METABOLISM	1.46	0.049	0.138