

Nuclear Respiratory Factor 1 Associated with Prognosis and Immune Infiltration in Hepatocellular Carcinoma

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Abstract

Background: Recent studies have shown that functional mitochondria are essential for cancer cells. Nuclear respiratory factor 1 (NRF1) is a transcription factor that activates mitochondrial biogenesis and the expression of the respiratory chain, but little is known about its prognostic value and tumor-infiltrating lymphocytes association. Here, we evaluated the association among expression of NRF1, clinicopathological characteristics, survival and immune infiltration in hepatocellular carcinoma (HCC).

Methods: We used the Tumor Immune Estimation Resource (TIMER) to analyze the difference of NRF1 mRNA expression in human cancers. Clinical-pathological information and follow-up data were collected from HCC (n = 171) and chronic hepatitis (n = 113) patients. NRF1 expression were scored based on the percentage and intensity of immunohistochemical staining in pathological slides. Correlations between clinical features and the expression of NRF1 were evaluated by Chi-square test, Kaplan-Meier curves, logrank tests and multivariate Cox regression analysis. The correlations between NRF1 expression and gene marker sets of tumor infiltrating lymphocytes (TILs) were analyzed by TIMER and Gene Expression Profiling Interactive Analysis (GEPIA) databases.

Results: NRF1 mRNA expression was significantly higher in HCC than in normal tissue. Compared with chronic hepatitis, more frequency of NRF1 high expression are found in HCC (31.58 % vs 13.27 %, $P < 0.001$, $P < 0.001$). In addition, the NRF1 expression was significantly associated with hepatic cirrhosis ($P = 0.021$) and vascular invasion ($P = 0.025$). NRF1 expression was also a significant independent predictor of survival in HCC ($P = 0.003$; $HR_{adj} = 0.20$; 95% CI = 0.09 – 0.44). NRF1 showed positively correlated with TILs, including B cell ($r = 0.384$, $P = 1.68e-13$), CD8+ T cells ($r = 0.246$, $P = 3.99e-06$), CD4+ T cells ($r = 0.535$, $P = 6.90e-27$), macrophage ($r = 0.506$, $P = 1.52e-23$), neutrophils ($r = 0.465$, $P = 6.08e-20$) and dendritic cell ($r = 0.404$, $P = 8.61e-15$). The marker genes of TILs correlated significantly with NRF1 expression.

Conclusions: NRF1 expression was a useful independent prognostic factor and correlated with tumor immune infiltration in HCC.

Background

Liver cancer is the second leading cause of cancer death worldwide in men [1]. Most primary liver cancer occurring worldwide is hepatocellular carcinoma (HCC) [2, 3]. The early diagnosis of HCC is so far complicated, and there is few effective therapies for HCC [4, 5]. Thus, an effective biomarker is urgently needed to estimate the prognosis. Recently instead of chemotherapies and targeted therapies, the immunotherapies have opened a new era of anticancer treatment. Given HCC is a prototype of inflammation-associated cancer, immunotherapies are the promising breakthroughs of anticancer treatment [6].

Hepatocytes which are rich in mitochondria, have developed diverse mechanisms to maintain mitochondrial homeostasis by regulating mitochondrial dynamics, biogenesis and degradation [7, 8]. The

emerging studies have shown that functional mitochondria are essential for the cancer cell [9]. Beyond the classical role in energy and metabolic mechanisms, mitochondria produce reactive oxygen species, which is capable of increasing tumorigenesis by activating signaling pathways that regulate cellular proliferation, metabolic alterations, and angiogenesis [10]. Mitochondria in cancer cell are different from their normal counterparts in structure and function [11]. Besides, robust reports have proposed links between immune function and mitochondrial processes. Indeed, monogenetic disorders of mitochondrial components may manifest with immune dysfunction [12]. Nuclear respiratory factor 1 (NRF1) is a key transcription factor linked to machinery in mitochondrial biogenesis and the transcriptional expression of the respiratory chain [13, 14]. NRF1 also has been identified as a valuable biomarker for breast cancer diagnosis and prognosis [15]. However, the effect of NRF1 in HCC progression and tumor immunology remains unclear. The aim of our study was to investigate the effects of NRF1 expression on the prognosis and the relationship with tumor infiltrating lymphocytes (TILs) in HCC.

Methods

Study populations

A panel of formalin-fixed, paraffin-embedded HCC and chronic hepatitis tissues were excised from fresh surgical samples at the Affiliated Hospital of Nantong University from 2004 to 2010. Clinical-pathological features and follow-up data were: gender, age at diagnosis, differentiation, vessel invasion, TNM stage, HBV infection, tumor size, AFP value and cirrhosis. None of the patients received radiotherapy, chemotherapy, or immunotherapy prior to surgery. The overall survival duration was the interval from the date of first biopsy to the date of death from disease.

Immunohistochemistry (ihc)

The tissue microarray (TMA) slides from patients were received for NRF1 staining. The NRF1 staining were performed by Tissue Microarray System (Quick-Ray, UT06, UNITMA, Korea). Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded sections and arranged in the new recipient paraffin blocks. IHC analysis was performed as previously described [16]. The slides were incubated with the primary antibodies against NRF1 (Abcam, Cambridge, MA, USA) at 4 °C overnight.

Vectra 3 System (PerkinElmer, USA) was used to acquire and analyze the images. There are two parameters estimated: intensity (0 to 3 as negative, weak, moderate or strong) and percentage (0% to 100%). The final staining score of each tissue sample was generated from intensity multiplied percentage scores. The cutoff value of NRF1 expression was set by X-tile software (<http://medicine.yale.edu/lab/rimm/research/software.aspx>; Rimm lab at Yale University).

Tumor Immune Estimation Resource (TIMER) and Gene Expression Profiling Interactive Analysis (GEPIA) Database Analysis

The level of NRF1 mRNA expression in different tumor types were obtained from TIMER (<https://cistrome.shinyapps.io/timer/>). TIMER was also employed to analyze the correlation between NRF1 mRNA expression and infiltration levels of immune cells in HCC [17, 18]. NRF1 was used for the y-axis, and other genes of interest are represented on the x-axis. The gene expression level was displayed with log2 RSEM. GEPIA (<http://gepia.cancer-pku.cn/index.html>) were employed to further confirm the results in TIMER. It is a web server for analyzing the RNA-Seq expression data from the TCGA and GTEx projects [19]. The Pearson method was used to determine the correlation coefficient. The tumor and normal tissue datasets were used for analysis.

Statistical Analyses

Correlations between clinicopathologic features and expression of NRF1 were evaluated by Chi-square test. Survival time was calculated from date of diagnosis to date of death/censoring. The log-rank test was used to assess differences between groups and the multivariate survival analysis was performed with Cox regression. All *P* values reported are from two-sided tests and the threshold for significance was set at *P*= 0.05. The statistical association and survival analyses were performed using STATA version 13.0 (StataCorp, TX, USA).

Results

The difference of NRF1 mRNA levels in human cancers and normal tissues

TIMER database showed that NRF1 mRNA expression was significantly higher in CHOL (bladder urothelial carcinoma), COAD (colon adenocarcinoma), KIRC (kidney renal clear cell carcinoma), KIRP(kidney renal papillary cell carcinoma), LIHC (liver hepatocellular carcinoma), while it was lower in BRCA(breast invasive carcinoma), LUAD (lung Adenocarcinoma), UCEC (uterine corpus endometrial carcinoma), PRAD (prostate adenocarcinoma) and THCA (thyroid carcinoma) compared with normal tissues (Fig. 1).

Since the TCGA database contains mRNA expression data, we used IHC to validate *in situ* protein expression in patient's tissue samples. Although low NRF1 expression accounted for the majority of HCC and non-tumor tissue (68.42% and 86.73%, respectively), more frequency of NRF1 high expression are found in HCC than in chronic hepatitis (31.58% vs 13.27%, *P*< 0.001) (Table 1).

Table 1
NRF1 expression in HCC and chronic hepatitis

	NRF1(Low)	NRF1(High)	Pearson χ^2	<i>P</i>
	n(%)	n(%)	12.39	0.000***
Chronic hepatitis	98(86.73)	15(13.27)		
HCC	117(68.42)	54(31.58)		
*** $P < 0.001$				

Association Between Nrf1 Expression And Clinicopathological Parameters In Hcc

In HCC patients, NRF1 expression presented a correlation with cirrhosis ($P = 0.021$) and vascular invasion ($P = 0.025$). By contrast, no correlation ($P > 0.05$ for all) was observed between NRF1 expression and other clinical parameters, such as sex, age at diagnosis, differentiation, TNM stage, tumor size and AFP value (Table 2).

Table 2
NRF1 expression and clinical variables in HCC

	NRF1(Low)	NRF1(High)	<i>P</i>
	n (%)	n (%)	
	117(68.42)	54(31.58)	
Gender			0.122
Female	26(59.09)	18(40.91)	
Male	91(71.65)	36(28.35)	
Age			0.189
< 55	71(72.45)	21(27.55)	
≥ 55	46(63.01)	27(36.99)	
Differentiation			0.203
1	9(50.00)	9(50.00)	
2	88(70.97)	36(29.03)	
3	19(67.86)	9(32.14)	
unknown	1(100.00)	0	
Vascular invasion			0.025*
No	61(61.62)	38(38.38)	
Yes	56(77.78)	16(22.22)	
TNM			0.497
I	41(63.08)	24(36.92)	
II	60(71.43)	24(28.57)	
III	16(72.73)	6(27.27)	
HBV			0.685
No	27(65.85)	14(34.15)	
Yes	90(69.23)	40(30.77)	
Tumor size			0.977
≤ 5 cm	66(68.04)	31(31.96)	
* <i>P</i> < 0.05			

	NRF1(Low)	NRF1(High)	<i>P</i>
	n (%)	n (%)	
> 5 cm	43(68.25)	20(31.57)	
unknown	8(72.73)	3(27.27)	
AFP			0.776
≤ 25 µg/L	37(66.07)	19(33.93)	
> 25 µg/L	52(68.42)	24(31.58)	
unknown	28(71.79)	11(28.21)	
Cirrhosis			0.021*
No	37(57.81)	27(42.19)	
Yes	80(74.77)	27(25.23)	
* <i>P</i> < 0.05			

Survival Analysis According To Nrf1 Expression For Hcc Patients

Kaplan–Meier survival curves revealed that HCC patients with high NRF1 expression had significantly better prognosis. While, low NRF1 expression showed worse survival ($P < 0.001$, Fig. 2). NRF1 ($P < 0.001$; HR = 0.20; 95% CI = 0.09–0.44), TNM stage ($P = 0.029$; HR = 1.53; 95% CI = 1.05–2.23) and tumor size ($P = 0.016$; HR = 1.84; 95% CI = 1.12–3.01) were associated with 5-year survival rate of HCC patients. After adjustment for clinical variables, NRF1 ($P = 0.003$; HR_{adj} = 0.20; 95% CI = 0.09–0.44) served as an independent prognosis factor (Table 3).

Table 3
Cox regression analysis of prognostic factors for 5-year survival in HCC

	Univariate analysis			Multivariate analysis		
	P	HR	95% CI	P	HR	95% CI
Gender	0.847	0.95	0.54–1.67			
male vs female						
Age	0.506	0.84	0.50–1.41			
< 55 vs ≥ 55						
Differentiation	0.911	0.97	0.62–1.54			
1 & 2 vs 3						
Vessel invasion	0.154	1.43	0.87–2.36			
no vs yes						
TNM	0.029*	1.53	1.05–2.23	0.697	1.09	0.70–1.72
I vs II & III						
HBV	0.682	1.21	0.49–3.02			
no vs yes						
Tumor size	0.016*	1.84	1.12–3.01	0.060	1.76	0.98–3.19
≤ 5 cm vs > 5 cm						
AFP	0.219	1.41	0.82–2.44			
≤ 25 vs > 25 µg/L						
Cirrhosis	0.088	1.68	0.93–3.04			
no vs yes						
NRF1	0.000***	0.20	0.09–0.43	0.003**	0.20	0.09–0.44
low vs high						
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$						

NRF1 expression correlate with the infiltration levels of immune cells in HCC

The survival times of patients in several cancers is determined by the quantity and activity status of TILs [20, 21]. Therefore, we investigated whether NRF1 expression was correlated with immune infiltration levels in HCC. Data from TIMER datasets demonstrated that NRF1 expression had significant correlations

with infiltrating levels of B cell ($r = 0.384$, $P = 1.68\text{e-}13$), CD8 + T cells ($r = 0.246$, $P = 3.99\text{e-}06$), CD4 + T cells ($r = 0.535$, $P = 6.90\text{e-}27$), macrophage ($r = 0.506$, $P = 1.52\text{e-}23$), neutrophils ($r = 0.465$, $P = 6.08\text{e-}20$) and dendritic cell ($r = 0.404$, $P = 8.61\text{e-}15$) (Fig. 3A).

Given the influence of tumor purity on immune infiltration analysis, the correlation analysis was adjusted for purity [20]. We determined the relationship between NRF1 and the diverse immune infiltrating cells based on the levels of immune marker gene expression by TIMER (Table 4, Fig. 3B). Specifically, NRF1 expression showed significant correlation with the immune markers such as B cells markers, CD19 ($r = 0.251$; $P = 2.39\text{e-}06$) and CD79A ($r = 0.273$; $P = 2.60\text{e-}07$), monocyte markers CD86 ($r = 0.407$; $P = 3.43\text{e-}15$) and CD115 ($r = 0.368$; $P = 1.73\text{e-}12$), TAM markers, CCL2 ($r = 0.298$; $P = 1.74\text{e-}08$), CD68 ($r = 0.264$; $P = 6.47\text{e-}07$) and IL10 ($r = 0.353$; $P = 1.48\text{e-}11$), macrophage markers, IRF5 ($r = 0.533$; $P = 9.18\text{e-}27$), COX2 ($r = 0.394$; $P = 2.89\text{e-}14$), CD163 ($r = 0.216$; $P = 5.38\text{e-}05$), VSIG4 ($r = 0.229$; $P = 1.73\text{e-}05$) and MS4A4A ($r = 0.203$; $P = 1.43\text{e-}04$), neutrophils markers, CD11b ($r = 0.354$; $P = 1.3\text{e-}11$), CCR7 ($r = 0.269$; $P = 4.13\text{e-}07$), dendritic cell markers, HLA-DPB1 ($r = 0.266$; $P = 5.48\text{e-}07$), HLA-DQB1 ($r = 0.196$; $P = 2.52\text{e-}04$), HLA-DRA ($r = 0.244$; $P = 4.63\text{e-}06$), HLA-DPA1 ($r = 0.274$; $P = 2.35\text{e-}07$), BDCA-1 ($r = 0.255$; $P = 1.57\text{e-}06$), BDCA-4 ($r = 0.542$; $P = 9.17\text{e-}28$), CD11c ($r = 0.435$; $P = 2.26\text{e-}17$). The NRF1 expression correlated significantly with the expression of the marker genes in different subsets of T cells, namely, Th1 markers, STAT4 ($r = 0.281$; $P = 1.13\text{e-}07$), STAT1 ($r = 0.41$; $P = 2.04\text{e-}15$), IFN- γ ($r = 0.241$; $P = 5.780\text{e-}06$) and TNF- α ($r = 0.401$; $P = 8.92\text{e-}15$), Th2 markers, GATA3 ($r = 0.385$; $P = 1.33\text{e-}13$), STAT6 ($r = 0.365$; $P = 2.74\text{e-}12$) and STAT5A ($r = 0.523$; $P = 1.23\text{e-}25$), Tfh marker, BCL6 ($r = 0.441$; $P = 7.59\text{e-}18$), Th17 marker, STAT3 ($r = 0.312$; $P = 3.29\text{e-}09$), Treg markers, CCR8 ($r = 0.421$; $P = 2.89\text{e-}16$), STAT5B ($r = 0.51$; $P = 2.88\text{e-}28$), and exhausted T cell markers, PD-1 ($r = 0.383$; $P = 1.83\text{e-}13$), CTLA4 ($r = 0.323$; $P = 7.77\text{e-}10$), LAG3 ($r = 0.256$; $P = 1.47\text{e-}06$) and TIM-3 ($r = 0.421$; $P = 2.99\text{e-}16$).

Table 4
Correlation analysis between NRF1 expression and markers of immune cells using
TIMER.

Description	Gene markers	None		Purity	
		Cor	P	Cor	P
CD4 + T cell	CD4	0.192	1.96e-04*	0.274	2.35e-07**
CD8 + T cel	CD8A	0.158	2.23e-03	0.267	5.08e-07**
	CD8B	0.12	2.09e-02	0.216	5.10e-05*
T cell (general)	CD3D	0.155	2.72e-03	0.26	9.99e-07**
	CD3E	0.137	8.33e-03	0.278	1.58e-07**
	CD2	0.141	6.54e-03	0.271	3.21e-07**
B cell	CD19	0.193	1.85e-04*	0.251	2.39e-06**
	CD79A	0.159	2.17e-03	0.273	2.60e-07**
Monocyte	CD86	0.266	2.31e-07**	0.407	3.43e-15***
	CD115(CSF1R)	0.223	1.56e-05*	0.368	1.73e-12***
TAM	CCL2	0.177	6.36e-04	0.298	1.74e-08***
	CD68	0.176	6.54e-04	0.264	6.47e-07**
	IL10	0.239	3.22e-06**	0.353	1.48e-11***
M1 Macrophage	INOS(NOS2)	0.083	1.12e-01	0.087	1.06e-01
	IRF5	0.536	5.23e-29***	0.533	9.18e-27***
	COX2(PTGS2)	0.394	2.89e-14***	0.394	2.89e-14***
M2 Macrophage	CD163	0.109	3.63e-02	0.216	5.38e-05*
	VSIG4	0.115	2.68e-02	0.229	1.73e-05*
	MS4A4A	0.091	8.16e-02	0.203	1.43e-04*
Neutrophils	CD66b(CeACAM8)	0.056	2.84e-01	0.083	1.23e-01
	CD11b(ITGAM)	0.277	6.9e-08**	0.354	1.3e-11***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Description	Gene markers	None		Purity	
	CCR7	0.14	7.02e-03	0.269	4.13e-07**
Natural killer cell	KIR2DL1	0.051	3.26e-01	0.03	5.84e-01
	KIR2DL3	0.189	2.58e-04*	0.229	1.72e-05*
	KIR2DL4	0.133	1.06e-02	0.163	2.37e-03
	KIR3DL1	0.069	1.87e-01	0.102	5.88e-02
	KIR3DL2	0.13	1.21e-02	0.186	5.25e-04
	KIR3DL3	0.052	3.17e-01	0.041	4.51e-01
	KIR2DS4	0.097	6.13e-02	0.091	9.17e-02
Dendritic cell	HLA-DPB1	0.162	1.81e-03	0.266	5.48e-07**
	HLA-DQB1	0.104	4.46e-02	0.196	2.52e-04*
	HLA-DRA	0.144	5.46e-03	0.244	4.63e-06*
	HLA-DPA1	0.165	1.49e-03	0.274	2.35e-07**
	BDCA-1(CD1C)	0.176	6.62e-04	0.255	1.57e-06**
	BDCA-4(NRP1)	0.507	0e + 00***	0.542	9.17e-28***
	CD11c(ITGAX)	0.318	4.3e-10***	0.435	2.26e-17***
Th1	T-bet(TBX21)	0.098	5.93e-02	0.19	3.77e-04
	STAT4	0.217	2.71e-05*	0.281	1.13e-07**
	STAT1	0.364	6.19e-13***	0.41	2.04e-15***
	IFN-γ(IFNG)	0.17	1.04e-03	0.241	5.78e-06*
	TNF-α(TNF)	0.284	2.56e-08***	0.401	8.92e-15***
Th2	GATA3	0.238	3.67e-06**	0.385	1.33e-13***
	STAT6	0.371	1.45e-13***	0.365	2.74e-12***
	STAT5A	0.456	1.71e-20***	0.523	1.23e-25***
	IL13	0.128	1.37e-02	0.121	2.44e-02

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Description	Gene markers	None		Purity	
Tfh	BCL6	0.425	0e-00***	0.441	7.59e-18***
	IL21	0.074	1.57e-01	0.11	4.18e-02
Th17	STAT3	0.267	1.91e-07**	0.312	3.29e-09***
	IL17A	0.102	4.92e-02	0.114	3.39e-02
Treg	FOXP3	0.136	8.52e-03	0.177	9.93e-04
	CCR8	0.331	5.87e-11***	0.421	2.89e-16***
	STAT5B	0.526	0e + 0	0.51	2.88e-28***
T cell exhaustion	PD-1(PDCD1)	0.284	2.48e-08***	0.383	1.83e-13***
	CTLA4	0.222	1.64e-05*	0.323	7.77e-10***
	LAG3	0.21	4.92e-05*	0.256	1.47e-06**
	TIM-3(HAVCR2)	0.274	9.08e-08**	0.421	2.99e-16***
	GZMB	0.093	7.47e-02	0.148	5.79e-03
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$					

As expect, the results obtained from GEPIA were consistent with the TIMER analysis results (Table 5). These findings strongly suggested that NRF1 was specifically correlated with TILs in HCC.

Table 5
Correlation analysis between NRF1 expression and markers of immune cells using GEPIA.

Description	Gene markers	Normal		Tumor	
		Cor	P	Cor	P
T cell (general)	CD3D	0.41	0.0029**	0.18	0.00065***
	CD3E	0.46	0.00086***	0.2	8e-05
	CD2	0.43	0.0021**	0.22	2.2e-05*
B cell	CD19	0.31	0.026*	0.17	0.00089***
	CD79A	0.38	0.0058**	0.15	0.0034**
Monocyte	CD86	0.66	1.5e-07**	0.35	3.2e-12***
	CD115(CSF1R)	0.49	0.00034***	0.33	4.2e-11***
TAM	CCL2	0.52	0.00012***	0.25	1.2e-06**
	CD68	0.68	6.4e-08***	0.26	4.8e-07**
	IL10	0.34	0.016*	0.23	5.3e-06***
M1 Macrophage	INOS(NOS2)	0.083	1.12e-01	0.028	0.59
	IRF5	0.61	2.5e-06**	0.57	0***
	COX2(PTGS2)	0.48	0.00047***	0.3	6.4e-09***
M2 Macrophage	CD163	0.43	0.0017**	0.18	0.00059***
	VSIG4	0.4	0.0044**	0.24	2.8e-06**
	MS4A4A	0.56	2.3e-05*	0.22	2.6e-05*
Neutrophils	CD66b(CeACAM8)	0.066	0.65	-0.0052	0.92
	CD11b(ITGAM)	0.57	1.4e-05*	0.42	0***
	CCR7	0.53	6.7e-05	0.17	0.00082***
Dendritic cell	HLA-DPB1	0.65	2.7e-07**	0.24	2.9e-06**
	HLA-DQB1	0.37	0.0091**	0.39	0.46

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Description	Gene markers	Normal		Tumor	
	HLA-DRA	0.6	3.6e-06**	0.22	1.4e-05**
	HLA-DPA1	0.6	3.9e-06**	0.21	4.9e-05*
	BDCA-1(CD1C)	0.47	0.00061***	0.27	1.2e-07**
	BDCA-4(NRP1)	0.53	7e-05	0.51	0***
	CD11c(ITGAX)	0.65	3.3e-07**	0.31	1.6e-09***
Th1	T-bet(TBX21)	0.58	8.9e-06*	0.15	0.005**
	STAT4	0.59	8.7e-06*	0.24	3e-06**
	STAT1	0.21	0.15	0.29	1e-08***
	IFN-(IFNG)	0.53	8.2e-05	0.13	0.014*
	TNF-A(TNF)	0.49	0.00026***	0.33	1.6e-10***
Th2	GATA3	0.33	0.02*	0.31	2.2e-09***
	STAT6	0.74	8.6e-10***	0.43	0***
	STAT5A	0.72	5.1e-09***	0.38	3.5e-14***
	IL13	0.068	0.64	0.0099	0.85
Tfh	BCL6	0.29	0.04*	0.31	8e-10***
	IL21	-0.33	0.82	0.071	0.17
Th17	STAT3	0.27	0.071	0.4	4.4e-16***
	IL17A	0.14	0.32	0.36	0.49
Treg	FOXP3	0.0054	0.97	-0.0073	0.89
	CCR8	0.43	0.0018**	0.2	7.3e-05
	STAT5B	0.8	4.6e-12***	0.49	0***
T cell exhaustion	PD-1(PDCD1)	0.43	0.0021**	0.24	2.8e-06**
	CTLA4	0.37	0.0087**	0.15	0.0034***
	LAG3	0.3	0.036*	0.13	0.014*
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$					

Description	Gene markers	Normal		Tumor	
	TIM-3(HAVCR2)	0.63	1.1e-06**	0.24	3.1e-06**
	GZMB	0.51	0.00016***	0.074	0.16
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$					

Discussion

It is clear that the biology of mitochondria in cancer are important to our understanding of cancer biology, as many classical cancer hallmarks result in altered mitochondrial function in tumor [22, 23]. NRF1 serves as an activator that promotes mitochondria biogenesis and helps support mitochondrial function [23, 24]. Furthermore, it has been identified that NRF1 binding motifs presented in many genes which operated in signaling pathways governing all hallmarks of malignant transformation and progression. The novel roles of NRF1 has been reported in cancer development and progression through its interplay with the transcription factors E2F4 and MYC [25]. Thus, NRF1 inevitably need to be taken into account when evaluating prognostics and therapeutic options for cancer patients. In our study, we demonstrated that NRF1 had correlation with some clinical variables in HCC, such as cirrhosis and vascular invasion. In the survival analysis, NRF1 functioned independently as a prognostic factor for HCC patients.

Tumor immunotherapy is a promising and transformative therapeutic strategy for patients with advanced cancers [26, 27]. Our data also demonstrated that NRF1 expression correlated with the infiltration status of B cells, CD4 + and CD8 + T cells, macrophages, neutrophils, and DCs. It suggested that NRF1 involved in regulating tumor immunity, and therefore influenced HCC prognosis. T cells have been key mediators of antitumor function in cancer immunotherapy and checkpoint blockade therapies have shown potent therapeutic effects in advanced cancer [27, 28]. We observed that expression of exhausted T cells markers, PD-1, CTLA-4 and TIM-3, which are critical inhibitory immune checkpoint proteins positively correlated with NRF1 expression. This suggested that NRF1 may inhibit or promote immune cells infiltrating, although the underlying mechanism is unknown.

Several limitations could influence the outcomes of this study. Firstly, the analysis of infiltration immune correlation is based RNA-seq data retrieved from in public repositories. Hence, the quality of data can influence the study outcomes. However, we did not verify TILs outcomes by testing our own clinical samples. Secondly, the sample sizes of HCC patients in our study were somehow small. Larger sample size will be necessary for reliable interpretation of data. Thirdly, racial or ethnic differences that are not explained or discussed in our study.

Conclusion

To summarize, NRF1 is a valuable biomarker for HCC, because it was associated with prognosis and immune cell infiltration. We proposed a rationale for future studies to develop NRF1 signaling-based

therapeutics to target HCC.

Abbreviations

NRF1

nuclear respiratory factor 1

HCC

hepatocellular carcinoma

TILs

tumor infiltrating lymphocytes (TILs)

TIMER

Tumor Immune Estimation Resource

GEPIA

Gene Expression Profiling Interactive Analysis

CHOL

bladder urothelial carcinoma)

COAD

colon adenocarcinoma

KIRC

kidney renal clear cell carcinoma

KIRP

kidney renal papillary cell carcinoma

LIHC

liver hepatocellular carcinoma

BRCA

breast invasive carcinoma

LUAD

lung Adenocarcinoma

UCEC

uterine corpus endometrial carcinoma

PRAD

prostate adenocarcinoma

THCA

thyroid carcinoma

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Human Research Ethics Committee of the Affiliated Hospital of Nantong University (2017-K036). Written informed consent was obtained from the

patients for publication of this study.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DW and LZ conceived the study. XJZ and PPS stained the tumors. LLH, DW and YPL performed the analyses, interpreted the data. DW and LLH wrote the paper. LZ gave critical appraisal of the manuscript.

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Figures

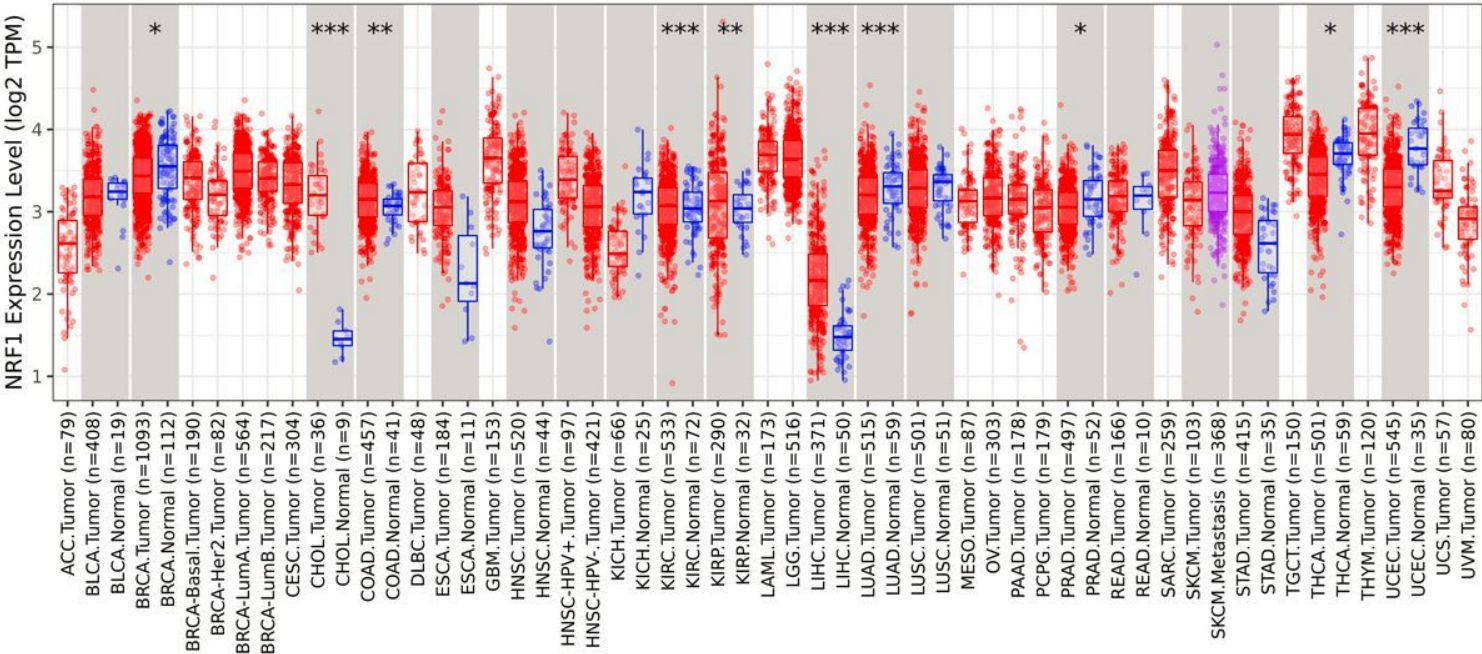
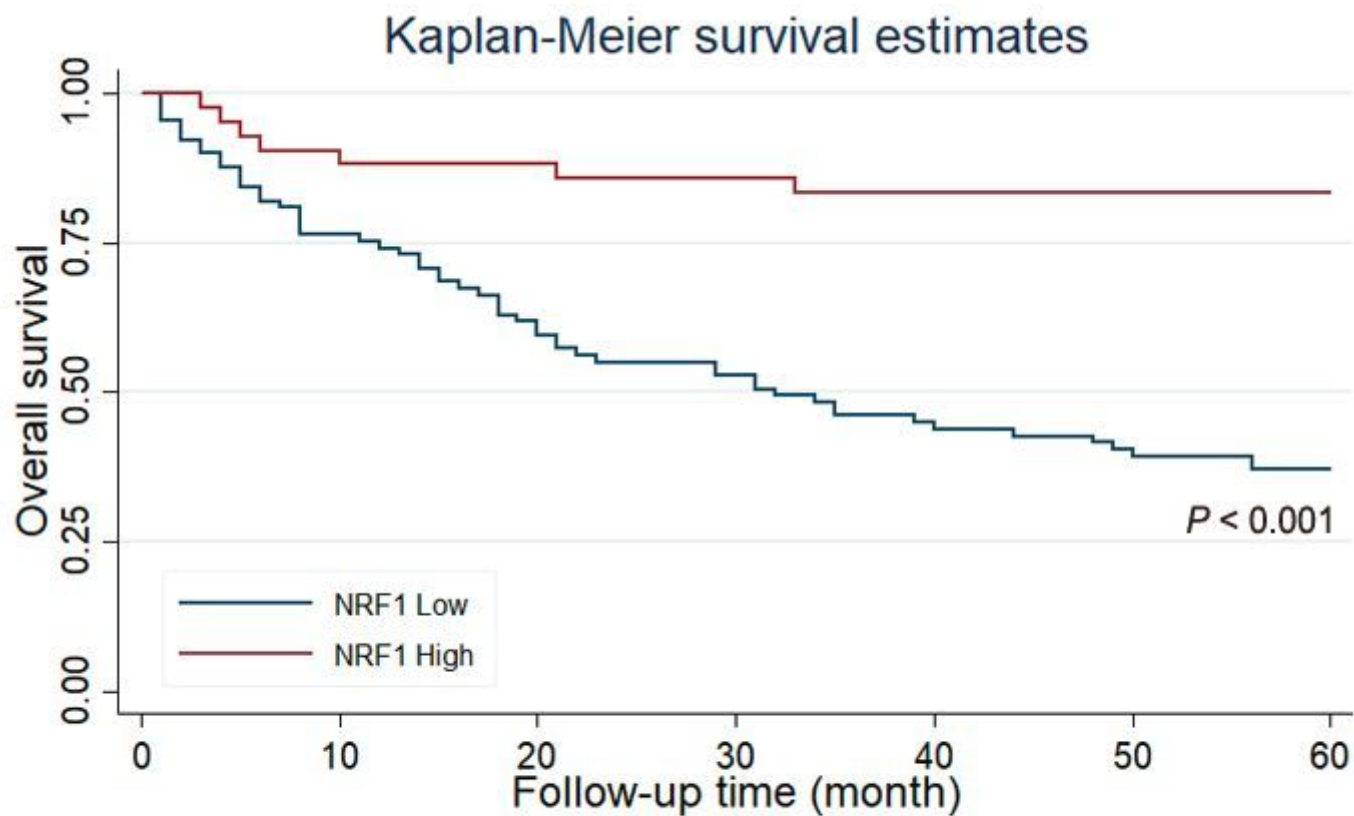


Figure 1

NRF1 mRNA expression in HCC and normal tissue. The level of NRF1 mRNA expression in different tumor types were obtained from TIMER database. *P < 0.05, **P < 0.01, ***P < 0.001.



Low (89)	21	15	6	8	4	35
High (42)	5	0	1	1	0	35

Figure 2

Kaplan-Meier survival curves of NRF1 expression in HCC. NRF1 high expression levels versus low expression levels were compared.

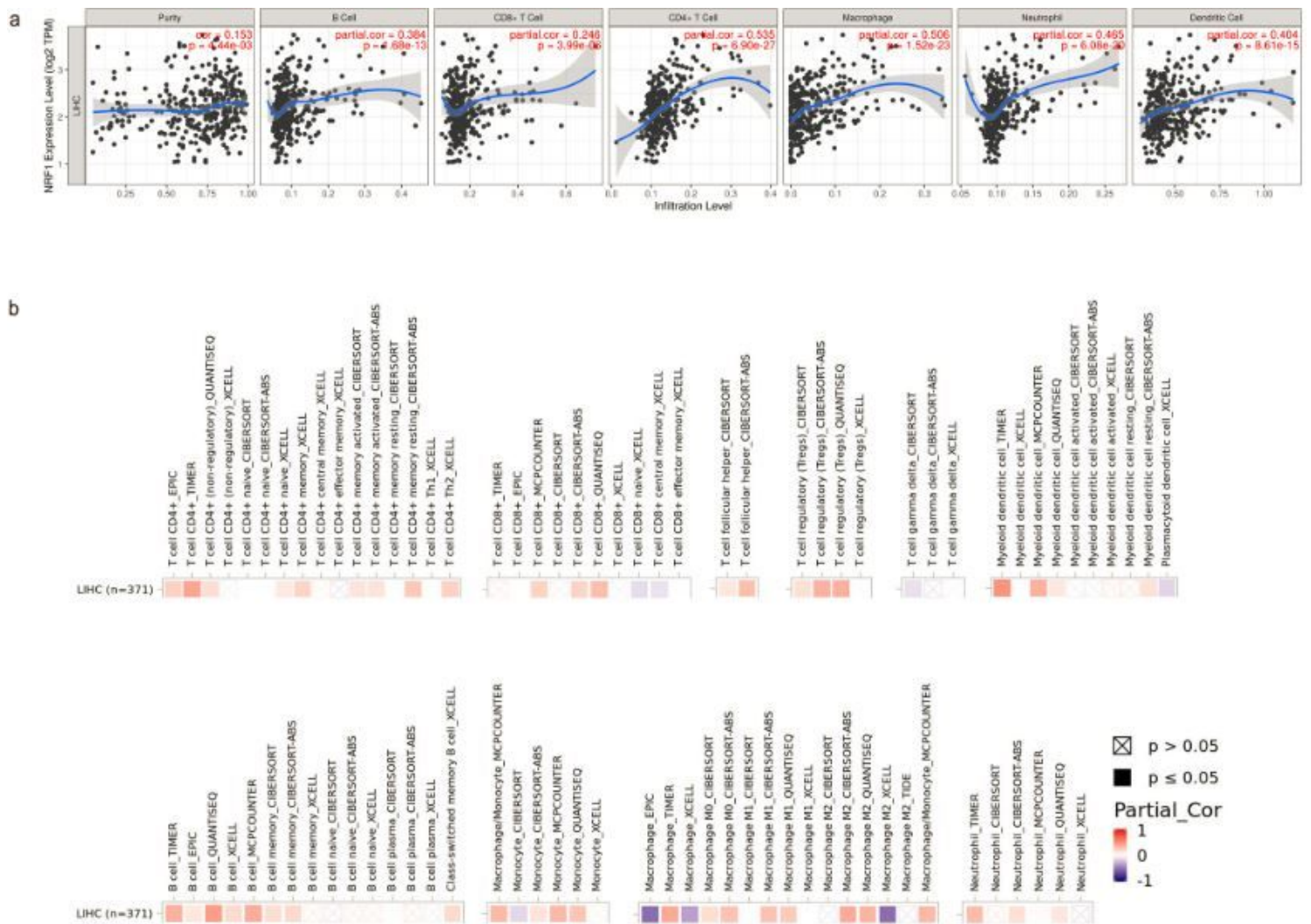


Figure 3

Correlation between NRF1 expression and immune cell infiltration in HCC. (A) Tumor purity, B cell, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells relative to NRF1 expression based on TIMER database. (B) CD4+ T cell, CD8+ T cell, T follicular helper cell, regulatory T cell, $\gamma\delta$ T cell, myeloid dendritic cell, B cell, monocyte, macrophage, and neutrophil relative to NRF1 expression based on TIMER2.0 database.