N6-methyladenosine binding protein YTHDF2 predicts better prognosis in patients with gastric cancer

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Primary research

Keywords: Gastric cancer, YTH N6-methyladenosine RNA binding protein 2 (YTHDF2), the Cancer Genome Atlas (TCGA), propensity score matching, prognosis

Posted Date: October 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-98084/v1>

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Abstract

Background: The potential role of N6-methyladenosine (m6A) in cancer progression has received tremendous attention over the past few years. The aim of this study was to evaluate the effect of YTH N6-methyladenosine RNA binding protein 2 (YTHDF2) on the prognosis of patients and its potential role in gastric cancer.

Methods: A total of 305 gastric cancer patients with clinical information were identified from the TCGA dataset. Limma package was used to analyze the differential m6A regulators; the Cox regression model was used to determine the risk factor for OS. A 1:1 propensity score matching (PSM) analysis was employed to adjust for the difference in baseline clinicopathological characteristics between the YTHDF2 low and high expression group. The Cox regression analysis was reused to identify the risk factors for overall survival (OS). GO and KEGG analysis were used to explore the potential role and function of YTHDF2 in gastric cancer.

Results: Nineteen m6A methylation regulators were expressed in gastric cancer tissues; YTHDF2 was associated with the prognosis of gastric cancer patients. The expression level of YTHDF2, patient age, and tumor stage were independent risk factors for OS. After PSM, YTHDF2 expression led to a relatively better prognosis and stage. Patients in stage IV had a significantly poor prognosis. The expression of YTHDF2 was associated with cancer-related functions and pathways in gastric cancer.

Conclusions: The high expression of YTHDF2 can predict a better prognosis of gastric cancer patients. YTHDF2 exerts a critical role in gastric cancer progression.

Background

Gastric cancer (GC) is one of the most common malignancies, and the third most common cause of cancer-related death worldwide [1]. Because early symptoms are not obvious, most patients are often diagnosed at an advanced stage. Together with the rapid progression and metastasis of GC, the prognosis of patients is dismal [2]. The lack of precise treatment and evaluation strategies has encouraged researchers to explore the pathogenesis and risk factors of GC in order to assess survival and propose new clinical treatment strategies. Exploring the risk factors and biomarkers affecting the prognosis of GC patients and proposing appropriate treatment methods is a prospective method for improving the prognosis in patients with various stages of GC.

N6-methyladenosine (m6A) is one of the most common RNA modifications. The potential role of m6A in cancer progression has received tremendous attention over the past few years [3]. The occurrence of m6A modification is initiated by m6A methyltransferase called "writer", removed by demethylase called "eraser" and recognized by m6A binding protein called "reader" [4, 5]. M6A "readers" bind m6A YTH domain-containing proteins and target different downstream RNAs [6, 7]. YTH N6-methyladenosine RNA binding protein 2 (YTHDF2) often leads to mRNA degradation by recognizing m6A in the 3'-UTR of the mRNA. Additionally, YTHDF2 can retain m6A methylation in the 5'-UTR and promote protein translation [8].

Recently, it has been reported that YTHDF2 can help LINC00470-METTTL3 mediated PTEN mRNA degradation in GC [9]. Also, knockdown of YTHDF2 can inhibit proliferation and promote apoptosis in the MGC803 GC cell line [10]. However, the relationship between YTHDF2 and prognosis of GC patients remains unclear.

In this study, we evaluated the effect of YTHDF2 on the prognosis of patients and its potential roles in GC.

Methods

Data source and acquisition

The expression data of GC and normal tissues were collected from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov>). The clinical information from TCGA was downloaded from the UCSC Xena database (<http://xena.ucsc.edu>). We collected 375 tumor tissues and 32 normal tissues expression data and retained valid clinical information of 305 GC patients. The TCGA dataset was normalized by log-transforming the Fragment Per Kilobase Per Million Reads (FPKM) +1.

m6A methylation regulators

According to the existing publications, we selected 22 m6A methylation regulators for screening risk factors affecting the prognosis of GC, including 7 m6A writers (KIAA1429, METTL14, METTL3, METTL4, RBM15, RBM15B, WTAP), 14 m6A readers (DGCR8, EIF3A, EIF3B, ELAVL1, HNRNPA2, HNRNPB1, HNRNPC1, HNRNPC2, SRSF2, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3) and 2 m6A erasers (ALKBH5 and FTO).

Statistical analysis

To determine the expression level of 22 m6A methylation regulators in tumor and normal tissues, the limma package was used for analysis. A Chi-square test was used to compare the distribution of clinicopathological features between patients with low and high expression of YTHDF2. The Cox regression model was chosen to identify m6A regulators and independent prognostic factors for OS. Hazard ratios (HRs) and 95% confidence interval (95% CIs) were also determined. OS was estimated by the Kaplan-Meier method, with a log-rank test to determine statistical significance. Patients in the low and high expression levels of YTHDF2 were matched at a ratio of 1:1 through propensity score matching (PSM); a total of 212 patients were included for subsequent analysis. GO and KEGG analysis of differentially expressed genes between YTHDF2 low and high expression groups were performed using the cluster Profiler package. SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) and R software for windows version R-4.0.2 (The R Foundation for Statistical Computing, Vienna, Austria) were used for data analysis. A P-value < 0.05 was considered statistically significant.

Results

The m6A methylation regulators expression level in GC

We performed the analysis of differentially expressed genes in 35 normal tissues and 375 tumor tissues downloaded from the TCGA database. As shown in **Figure 1A** and **1B**, 19 of 22 m6A methylation regulators were expressed in GC, among which, 17 m6A methylation regulators were significantly upregulated in GC tissues, including m6A “writer” (KIAA1429, METTL14, METTL3, METTL4, RBM15, RBM15B, WTAP) and “reader” (DGCR8, EIF3A, EIF3B, ELAVL1, SRSF2, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3).

The YTHDF2 expression level in baseline clinicopathological characteristics

To understand the relationship between 19 m6A methylation regulators and patient prognosis, we performed univariate Cox regression analysis for 19 regulators, respectively. We found that m6A “reader” YTHDF2 was obviously associated with the OS (**Additional file 1**). The results also indicated that YTHDF2 might perform as a protective factor role in GC (HR = 0.629, p = 0.027). Consequently, GC patients were divided into low and high expression groups according to the expression level of YTHDF2 in GC tissues (**Figure 2A**). Combining the baseline clinicopathological characteristics of the patients, we found that gender and subtype were both related to the expression of YTHDF2 (**Table 1**). Yet, the YTHDF2 expression level was significantly higher in females than in males, while no difference was found in MSS, MSS-L, and MSS-H (**Figure 2B - I**).

Table 1 Baseline clinicopathological characteristics

Variables	Total (N=305)	YTHDF2 Low (N=152)	YTHDF2 High (N=153)	P value
Age, n (%)				0.122
≤65	139 (45.6)	76 (24.9)	63 (20.7)	
>65	166 (54.4)	76 (24.9)	90 (29.5)	
Gender, n (%)				0.049
female	113 (37.0)	48 (15.7)	65 (21.3)	
male	192 (63.0)	104 (34.1)	88 (28.9)	
Stage, n (%)				0.287
Stage I	39 (12.8)	22 (7.2)	17 (5.6)	
Stage II	99 (32.5)	53 (17.4)	46 (15.1)	
Stage III	135 (44.3)	59 (19.3)	76 (24.9)	
Stage IV	32 (10.5)	18 (5.9)	14 (4.6)	
Grade, n (%)				0.666
G1-2	112 (36.7)	54 (17.7)	58 (19.0)	
G3-4	193 (63.3)	98 (32.1)	95 (31.1)	
T, n (%)				0.219
T1-2	75 (24.6)	42 (13.8)	33 (10.8)	
T3-4	230 (75.4)	110 (36.1)	120 (39.3)	
M, n (%)				0.633
M0	285 (93.4)	141 (46.2)	144 (47.2)	
M1	20 (6.6)	11 (3.6)	9 (3.0)	
N, n (%)				0.098
N0	93 (30.5)	53 (17.4)	40 (13.1)	
N+	212 (69.5)	99 (32.5)	113 (37.0)	
Subtype, n (%)				<0.0001
MSS	204 (66.9)	119 (39.0)	85 (27.9)	
MSI-L	48 (15.7)	17 (5.6)	31 (10.2)	
MSI-H	53 (17.4)	16 (5.2)	37 (12.1)	

Risk factors of patient survival

To explore the risk factors that can influence the prognosis of GC patients in baseline clinicopathological characteristics, univariate and multivariate Cox regression analyses were used for determining the risk factors for OS. As a result, YTHDF2 expression, patient age, and tumor stage were confirmed as significant predictive factors for OS (**Table 2**). Patients with higher YTHDF2 expression had significantly better OS. Compared with patients younger than 65 years old, those > 65 years old were at high risk of worse OS. In terms of stage, an increased risk of poor prognosis was detected in stage III and IV, as compared with stage I and II.

Table 2 Cox regression analysis of prognostic factors for overall survival in gastric cancer (GC)

	Univariate analysis		Multivariate analysis	
	HR ^a (95% CI ^b)	P	HR (95% CI)	P value
YTHDF2 expression				
Low	Reference		Reference	
High	0.63 (0.42-0.95)	0.027	0.59 (0.39-0.89)	0.012
Age				
≤65	Reference		Reference	
>65	1.63 (1.08-2.46)	0.019	1.89 (1.24-2.88)	0.003
Gender				
female	Reference			
male	1.38 (0.89-2.12)	0.147		
Stage				
Stage I	Reference		Reference	
Stage II	1.78 (0.80-3.95)	0.156	1.82 (0.82-4.03)	0.138
Stage III	2.15(1.01-4.56)	0.047	2.32 (1.09-4.92)	0.029
Stage IV	3.70 (1.59-8.57)	0.002	4.23 (1.81-9.88)	<0.001
Grade				
G1-2	Reference			
G3-4	1.29 (0.86-1.95)	0.225		
T				
T1-2	Reference			
T3-4	1.51 (0.93-2.46)	0.096		
M				
M0	Reference			
M1	1.65 (0.80-3.40)	0.177		
N				
N0	Reference			
N+	1.43 (0.90-2.29)	0.130		
Subtype				

MSS ^c	Reference	
MSI-L ^d	1.40 (0.79-2.46)	0.246
MSI-H ^e	0.73 (0.42-1.28)	0.278

^aHR: hazard ratio; ^b95% CI: 95% confidence intervals; ^cMSS: microsatellite stability; ^dMSI-L: microsatellite instability-low; ^eMSI-H: microsatellite instability-high

YTHDF2 expression and patient survival

We further performed Cox regression analyses for each clinicopathological subgroup of GC patients (**Figure 3**), which revealed that patients younger than 65 years old, male patients, patients with T3-4, M0, N0, and MSS subtype had a better prognosis.

To ensure the accuracy of the risk factors obtained by our previous analysis, and to exclude the interference of other factors, we used propensity score matching (PSM) to match patients with low and high expression of YTHDF2 on a 1:1 basis. The probability of the YTHDF2 expression level was used as a propensity score, and the nearest neighbor optimal matching algorithm was used to find the best match for each patient. Successful matching was considered as the standard difference (SD) less than 0.2 (**Table 3**).

Table 3 Propensity score matching (PSM) in gastric cancer (GC) patients based on YTHDF2 expression

Variables	Before matching		SD ^a	After matching		SD
	Low (n=152)	High (n=153)		Low (n=106)	High (n=106)	
Age			0.178			0.095
≤65	76 (24.9)	63 (20.7)		49 (23.1)	44 (20.8)	
>65	76 (24.9)	90 (29.5)		57 (26.9)	62 (29.2)	
Gender			0.227			0.04
female	48 (15.7)	65 (21.3)		33 (15.6)	35 (16.5)	
male	104 (34.1)	88 (28.9)		73 (34.4)	71 (33.5)	
Stage			0.224			0.124
Stage I	22 (7.2)	17 (5.6)		11 (5.2)	14 (6.6)	
Stage II	53 (17.4)	46 (15.1)		35 (16.5)	34 (16.0)	
Stage III	59 (19.3)	76 (24.9)		48 (22.6)	49 (23.1)	
Stage IV	18 (5.9)	14 (4.6)		12 (5.7)	9 (4.2)	
Grade			0.049			<0.001
G1-2	54 (17.7)	58 (19.0)		38 (17.9)	38 (17.9)	
G3-4	98 (32.1)	95 (31.1)		68 (32.1)	68 (32.1)	
T			0.141			0.089
T1-2	42 (13.8)	33 (10.8)		23 (10.8)	27 (12.7)	
T3-4	110 (36.1)	120 (39.3)		83 (39.2)	79 (37.3)	
M			0.055			0.185
M0	141 (46.2)	144 (47.2)		96 (45.3)	101 (47.6)	
M1	11 (3.6)	9 (3.0)		10 (4.7)	5 (2.4)	
N			0.19			0.063
N0	53 (17.4)	40 (13.1)		29 (13.7)	32 (15.1)	
N+	99 (32.5)	113 (37.0)		77 (36.3)	74 (34.9)	
Subtype			0.502			0.12
MSS ^b	119 (39.0)	85 (27.9)		75 (35.4)	74 (34.9)	
MSI-L ^c	17 (5.6)	31 (10.2)		15 (7.1)	12 (5.7)	
MSI-H ^d	16 (5.2)	37 (12.1)		16 (7.5)	20 (9.4)	

^aSD: Standard Deviation; ^bMSS: microsatellite stability; ^cMSI-L: microsatellite instability-low; ^dMSI-H: microsatellite instability-high

Cox regression analysis was used to analyze the prognostic factors of patients with PSM. The high expression level of YTHDF2 and stage IV were independent risk factors affecting the prognosis of patients (**Table 4**). Moreover, survival analysis showed that patients with high expression of YTHDF2 had significantly better OS, and patients with stage IV had a worse OS than those with other stages (**Figure 4A** and **4B**).

Table 4 Cox regression analysis of prognostic factors for overall survival (OS) after matching

	HR ^a (95% CI ^b)	P
YTHDF2 expression		
Low	Reference	
High	0.58 (0.37-0.91)	0.018
Age		
≤65	Reference	
>65	1.37 (0.86-2.16)	0.184
Gender		
female	Reference	
male	1.79 (1.05-3.06)	0.034
Stage		
Stage I	Reference	
Stage II	1.32 (0.53-3.30)	0.548
Stage III	2.15 (0.90-5.10)	0.084
Stage IV	3.15 (1.16-8.55)	0.024
Grade		
G1-2	Reference	
G3-4	1.68 (1.04-2.71)	0.034
T		
T1-2	Reference	
T3-4	1.92 (1.05-3.50)	0.033
M		
M0	Reference	
M1	1.29 (0.56-2.98)	0.548
N		
N0	Reference	
N+	1.34 (0.79-2.28)	0.272
Subtype		
MSS ^c	Reference	

MSI-L ^d	1.64 (0.87-3.08)	0.124
MSI-H ^e	0.73 (0.39-1.37)	0.322

^aHR: hazard ratio; ^b95% CI: 95% confidence intervals; ^cMSS: microsatellite stability; ^dMSI-L: microsatellite instability-low; ^eMSI-H: microsatellite instability-high.

The potential role of YTHDF2 in GC

To explore the potential role of YTHDF2 in GC, we identified 3066 differential expression genes (DEGs) [$|\log_2(\text{Fold Change})| > 1$ and $p < 0.05$] between the low and high expression level of YTHDF2 (**Figure 4C** and **4D**). The Gene Ontology (GO) analyses showed the DEGs were mainly enriched in cancer- and methylation-associated biological process, cellular component and molecular function, such as histone modification, methylation, methyltransferase complex, histone methyltransferase complex, methyltransferase activity, and p53 binding (**Figure 5A** and **5B**). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analyses were primarily enriched in several cancer-associated pathways, such as cell cycle, RNA degradation, mismatch repair, viral carcinogenesis, apoptosis, and p53 signaling pathway.

Discussion

Over recent years, m6A methylation modification of RNA has received increasing attention. m6A was first described in the 1970s. It is the most abundant internal modifier of mRNA and long noncoding RNA (lncRNA) in most eukaryotes [11]. With the development of high-throughput sequencing technology, m6A was found to be mainly distributed in stop and 3'-UTR regions [12–14]. The dynamic regulation of m6A modification has shown to be significantly associated with the occurrence and development of complex human diseases, including the progression of tumors [6, 15, 16].

A previous study suggested that m6A “reader” YTHDF1 promotes gastric carcinogenesis by regulating the translation of FZD7 and is associated with poor prognosis of GC [17]. Moreover, “writer” METTL3 promotes GC cell proliferation, promotes or inhibits GC progression by regulating the expression of downstream target genes, regulates MYC signaling pathway, and promotes epithelial-mesenchymal transition (EMT) and GC metastasis [18–22]. As for other m6A regulators, FTO, ALKBH5, WTAP, and KIAA 1429 are involved in the progression of GC, thus affecting the prognosis of patients [23–25]. Regrettably, so far, only a few studies have reported on the regulatory function of YTHDF2 and its effect on prognosis in GC.

YTHDF2 has been associated with the prognosis of patients with a variety of tumors, including hepatocellular carcinoma, prostate cancer, and osteosarcoma [26–28]. In this study, we found that m6A methylation regulator YTHDF2 is an independent protective prognostic factor for OS [$p = 0.018$; HR (95% CI): 0.58 (0.37–0.91)] of patients with GC. In addition, multivariate analysis showed that patient age [$p = 0.003$; HR (95% CI): 1.89 (1.24–2.88)] and tumor stage [stage III: $p = 0.029$; HR (95% CI): 2.32 (1.09–4.92);

stage IV: $p < 0.001$; HR (95% CI): 4.23 (1.81–9.88)] were also independent factors affecting the prognosis. However, after the propensity score matching (PSM) was applied, the age no longer resulted as an independent prognostic factor [$p = 0.184$; HR (95% CI): 1.37 (0.86–2.16)], while GC stage, especially stage IV, was still a strong independent prognostic factor affecting OS [$p = 0.024$; HR (95% CI): 3.15 (1.16–8.55)]. Despite advances in clinical treatment over the past few decades, the prognosis of stage III and stage IV GC remains poor [29]. In this study, after PSM based on the expression of YTHDF2, stage III was no longer an independent prognostic risk factor for OS [$p = 0.084$; HR (95% CI): 2.15 (0.90–5.10)], but it still failed to change the poor prognosis of stage IV patients. Moreover, YTHDF2 was more likely expressed in female patients; but the difference in YTHDF2 expression between genders did not suggest it could affect the prognosis of patients.

Based on the functional analysis, we found that these differentially expressed genes were associated with a variety of tumor-related functions, including methylation, methyltransferase, cell cycle, RNA degradation, gene mismatch repair, apoptosis, and p53 signaling pathway. In addition, we also found that these genes are potentially associated with histone modification and histone methylation in GO analysis. In our previous study, we discovered that histone methyltransferase SETD1A interacts with HIF1 α to enhance glycolysis and promote GC progression [30]. Therefore, we believe that YTHDF2 has an important role in GC.

Our study has several limitations. The overall sample size is relatively small. Although we tried, we were not able to find data sets containing valid clinical information in databases such as Gene-Expression Omnibus (GEO). We have predicted and analyzed the prognosis and function of YTHDF2; yet, additional *in vivo* and *in vitro* experiments should be performed to verify the results.

Conclusions

Our study is the first study to predict m6A “reader” YTHDF2 as a protective prognosis factor in patients with GC. YTHDF2 may have a critical role in tumor regulation by regulating the expression of downstream target genes in GC.

Abbreviations

GC: gastric cancer; m6A: N6-methyladenosine; YTHDF2: YTH N6-methyladenosine RNA binding protein 2; TCGA: The Cancer Genome Atlas; OS: overall survival; FPKM: Fragment Per Kilobase Per Million Reads; HR: hazard ratio; 95% CI: 95% confidence interval; PSM: propensity score matching; SD: standard difference; DEGs: differential expression genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; lncRNA: long noncoding RNA; GEO: Gene-Expression Omnibus; MSS: microsatellite stability; MSI-L: microsatellite instability-low; MSI-H: microsatellite instability-high.

Declarations

Ethics approval and consent to participate

This study was approved by the institutional ethical review board of Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiao Tong University.

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available in the Cancer Genome Atlas repository (TCGA, <https://portal.gdc.cancer.gov>). All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Grant of Clinical Research Promotion Program of Shanghai Ninth People's Hospital, School of Medical, Shanghai Jiao Tong University (JYLJ201822).

Authors' contributions

JY designed the study. XX and CZ drafted the manuscript. XX, CZ and JZ collected and performed all data analysis. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by the Grant of Clinical Research Promotion Program of Shanghai Ninth People's Hospital, School of Medical, Shanghai Jiaotong University (JYLJ201822).

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Figures

Figure 1

The expression level of m6A RNA methylation regulators. (A) Expression differences of m6A RNA methylation regulators in gastric cancer based on TCGA data. Red and blue represent the relatively high or low expression, respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (B) Violin plot visualizing the differentially m6A RNA methylation regulators in gastric cancer (blue is normal and red is gastric cancer).

Figure 2

Analysis of YTHDF2 expression in patients with different baseline clinicopathological characteristics. (A) Patients were divided into low and high expression groups according to the expression level of YTHDF2. (B-I) Differences of YTHDF2 expression among age, gender, stage, grade, subtype, T, N, and M groups. *p < 0.05, NS means no significance.

Figure 3

Prognosis analysis in subtypes of each baseline clinicopathological characteristics group. The hazard ratios (HR) and 95% confidence intervals (CI) were calculated by univariate Cox regression.

Figure 4

The survival and differential expression genes analysis of YTHDF2 in gastric cancer. (A, B) The survival analysis between low and high YTHDF2 expression groups before propensity score matching (A) and after propensity score matching (B). (C) Expression differences of the top 30 differential expression

genes between low and high YTHDF2 expression groups. Red and blue represent the relatively high or low expression, respectively. (D) The volcano showed differential expression genes between low and high YTHDF2 expression groups.

Figure 5

The potential role and function of YTHDF2-related genes. Functional annotation of the differential expression genes between low and high YTHDF2 expression groups using GO (A, B) and KEGG pathway (C, D) analysis.

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