Observational Study

Medicine

OPEN

Clinical significance and prospective molecular mechanism of NUF2 in gastric cancer exploration

A comprehensive study based on the GeneChip, GEO, Oncomine, and TCGA databases

Lv'rong Ge[a], Qing Sun[b], Lian Xia[a], Xin Xu[a*]

Abstract

Nuf2 is a combination of silica and spindle microtubules during the cells in the cells, participating in regulatory cell proliferation and apoptosis. Previous studies have shown that the growth of gastric cancer (GC) cells is significantly inhibited after siRNA-mediated Nuf2 gene knockout. However, the expression, survival and molecular mechanism of nuf2 in patients with GC are still unclear.

This study revealed the prognostic role of Nuf2 in GC and its relationship with immune cells. The expression of Nuf2 in GC by TIMER database and Oncomine database, and evaluated the relationship between the expression of Nuf2 and the survival and prognosis of patients with GC by Kaplan–Meier Plotter database and gene expression profiling interactive analysis database.

Here, we revealed that Nuf2 is highly expressed in GC and is related to the prognosis of patients with GC. And there is a significant negative correlation between the Nuf2 transcription level and high immune cell infiltration. Notably, the expression of Nuf2 in GC patients with Her2 negative rather than positive is related to poor OS, FP and PPS, indicating the potential to target Nuf2 gene in GC patients with Her2 negative.

We suggested that Nuf2 could be used as a diagnostic gene as a biomarker of the occurrence and prognosis of GC.

Abbreviations: GC = gastric cancer, FP = first progression, OS = overall survival, PPS = postprogression survival, TNBC = triple-negative breast cancer.

Keywords: bioinformatic analysis, NUF2

1. Introduction

Gastric cancer (GC) is remain one of the most common cancers in the world.[1] In recent decades, the incidence of GC has declined rapidly worldwide,[2] but the reason is not completely clear. Part of the decline may be due to the identification of certain risk factors, such as *Helicobacter pylori* and other dietary and environmental risks.[3] Although the incidence is declining, the absolute number of new cases annually is growing, mainly due to the global population aging. In addition, due to some unknown reasons, the downward trend of morbidity has been interrupted, which has been replaced by the rising trend of young patients in recent years.[4] Serum tumor markers (including CEA and CA125) are limited to some patients. It is not a diagnostic inspection of the sensitivity and specificity of GC. Therefore, there is an urgent need to find a new biomarker for early screening of GC, and in-depth understanding of the molecular pathogenesis of GC is a prerequisite for controlling this disease.

The cell division-related gene Nuf2 (Nuf2 component of ndC80 kinetochore complex) is a combination of silica and spindle microtubules during the cells in the cells, participating in regulatory cell proliferation and apoptosis.[5] In the cell cycle, appropriate and accurate chromosomal separation is critical to maintaining the integrity of the genome, which requires appropriate coordination between chromosomes, dynamic particles, spindles, and NuF2 complexes as mitosis. The interaction and important checkpoints of spindle fittings play a main role of stabilizing granular-microtubules and ensuring the normal separation of chromosomes. When silent tumor cells are highly expressed Nuf2, the moving protein does not form an accessory of the spindle microtube, and the spindle body assembly checkpoint is not activated, causing abnormal separation of the chromosome to induce cell death in mitogenic cells.[6,7] It is reported that Nuf2 is overexpressed in different types of cancers, including lung cancer, colorectal cancer, liver cancer, ovarian cancer, and serous adenocarcinoma.[8–10] At present, studies have shown that the growth of GC cells is significantly inhibited after siRNA-mediated Nuf2 gene knockout. However, the expression, survival and molecular mechanism of nuf2 in patients with GC are still unclear.

In this study, we revealed the prognostic role of Nuf2 in GC and its relationship with immune cells. We analyzed the expression of Nuf2 in GC by TIMER database and Oncomine database, and

LG and QS have the same contributions.

The authors have no funding and conflicts of interest to disclose.

All data are available online from publicly available databases, please contact the authors if you need help.

[a] Department of Operating Room, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, China, [b] Department of Colorectal Surgery, Zhuji people's Hospital of Zhejiang Province, Shaoxing, China.

** Correspondence: Xin Xu, Department of Operating Room, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou 310016, China (e-mail: 3193136@zju.edu.cn).*

How to cite this article: Ge L, Sun Q, Xia L, Xu X. Clinical significance and prospective molecular mechanism of NUF2 in gastric cancer exploration: A comprehensive study based on the GeneChip, GEO, Oncomine, and TCGA databases. Medicine 2022;101:26(e29802).

Received: 25 August 2021 / Received in final form: 22 February 2022 / Accepted: 26 May 2022

http://dx.doi.org/10.1097/MD.0000000000029802

evaluated the relationship between the expression of Nuf2 and the survival and prognosis of patients with GC by Kaplan–Meier plotter database and gene expression profiling interactive analysis (GEPIA) database. The relationship between Nuf2 and tumor immune infiltration biomarkers was analyzed by TIMER and GEPIA. In this study, we revealed that Nuf2 is highly expressed in GC and is related to the prognosis of patients with GC. We suggested that Nuf2 can be used as a diagnostic gene as a biomarker of the occurrence and prognosis of GC and can be used in patients with GC.

2. Materials and methods

2.1. Oncomine database analysis

Oncomine database (www.oncomine.org) is a cancer profile database containing relevant tumor gene expression and clinical information.[11] We analyzed the transcriptome expression of Nuf2 in GC tissues and the corresponding normal tissues by Oncomine. When set Fold change >1.5 and P <.001, we think that there is a significant difference in its expression. The threshold of Generank is set to the top 10%, and the data type is "all."

2.2. TIMER database analysis

Tumor Immune Estimation Resource (TIMER) is a web database for systematical evaluations of the clinical significance of different immune cells types in 23 cancer types from TCGA database.[12] Using multiple immune deconvolution methods could provide immune infiltrates' abundances in tumor microenvironment. B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophage cells, neutrophil cells, and dendritic cells in the tumor microenvironment were defined as tumor-infiltrating immune cells.[13] Nuf2 expression levels in different tumors were analyzed in this study based on TIMER database.

2.3. Kaplan–Meier plotter analysis

Kaplan–Meier plotter (https://kmplot.com/analysis/) can evaluate the effect of 54K gene (mRNA, miRNA, protein) on the survival of 21 cancers, one of which is GC.[14] We performed an analysis for the correlation between Nuf2 expression and survival rate and clinical parameters in GC. Split patients by median as cutoff parameter and probe set options is user selected probe set.

2.4. GEPIA analysis

GEPIA is web-based tool for normal and tumor gene expression profiling and interactive analyses, providing critical interactive and customizable functions, such as differential expression analysis, correlation analysis, patient survival analysis, etc.[15] We used GEPIA database analyzing the relationship between Nuf2 expression and different immune cell biomarkers. Spearman method with default parameters were set in this analysis.

2.5. Institutional Review Board Statement

This article is dispensed from Institutional Review Board Given for the results in this study from public database.

3. Results

3.1. Expression of Nuf2 in GC

By using the Oncomine database, the expression level of Nuf2 in GC tissues is significantly higher than that of its normal tissue (Fig. 1A). In addition, the data of GC are analyzed in the TIMER database and the GEPIA database (Fig. 1B and C), indicating that Nuf2 may play a certain role in GC.

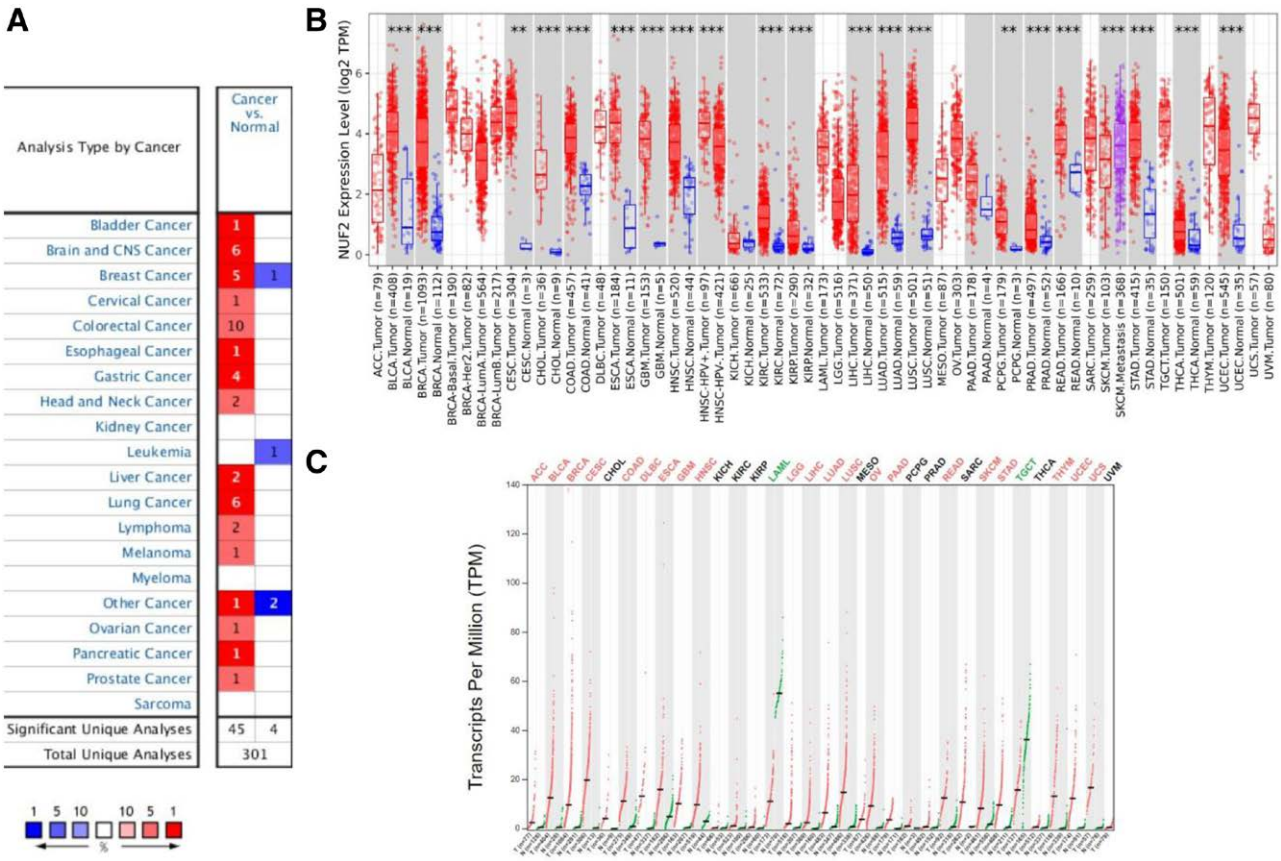

Figure 1. The expression of Nuf2 in tumor tissues. (A) Nuf2 expression in gastric tumor tissue compared to that in normal tissues (via Oncomine). (B) and (C) Nuf2 expression in different cancers (via TIMER and GEPIA) (***P < .001).

3.2. Prognostic role of Nuf2 in GC

The Kaplan–Meier plotter for GC provides detailed survival-related prognosis, including overall survival (OS), first progression (FP), and postprogression survival (PPS). The analysis showed that the OS of GC patients with low expression of Nuf2 was significantly worse than that of patients with high expression (Fig. 2A, hazard ratio [HR] = 0.67, 95% confidence interval [CI] = 0.54–0.84, P = .00036), and the possibility of FP was significantly higher than that of patients with high expression (Fig. 2B, HR = 0.71, 95% CI = 0.56–0.91, P = .0055). Interestingly, in postoperative patients with GC, patients with low expression of Nuf2 are also more likely to relapse after operation than those with high expression (Fig. 2C, HR = 0.54, 95% CI = 0.4–0.71, P = 9.1e–06). These results indicating that the low expression of NUF2 may lead to the progress of GC, which may be the potential prognostic biological marker of GC patients.

3.3. Correlations between Nuf2 and clinical characteristics in GC patients

Using the Kaplan–Meier plotter database, the relationship between expression of Nuf2 and diverse clinical characteristics in GC patients was analyzed. These clinical features contain gender, Her2 status, Lauren classification, N stage, and M stage. For the factor of gender, low expression of Nuf2 was associated with poor OS and PPS but not FP for GC patients. It is worth exploring that the expression of Nuf2 in GC patients with Her2-negative rather than positive is related to poor OS, FP, and PPS (Table 1). Notably, in terms of Lauren classification, Nuf2 expression was only associated with diffuse type but not intestinal type (Table 1). The expression level of Nuf2 was significantly correlated with OS, FP, and PPS when lymph node metastasis occurred in GC patients. While in the state of no metastasis, the expression level of Nuf2 in GC patients tends to be related to OS, FP, and PPS (Table 1). The differences of clinical factors indicate that the specific clinical conditions of patients should be considered when using Nuf2 as a biomarker.

3.4. Associations between Nuf2 and immune cell infiltration in GC

We used TIMER database to study the relationship between the expression of Nuf2 and the level of immune cell infiltration. In a nutshell, there was a significant negative correlation between the Nuf2 transcription level and high immune cell infiltration (Fig. 3). As shown in Figure 3, the expression of Nuf2 is significant negative associated with macrophages (R = 0.258, P = 3.43e–07), neutrophils (R = –0.178, P = 5.10e–04), B-cell infiltration (R = –0.116, P = 2.36e–02), CD4+ T cells (R = –0.166, P = 1.17e–03), CD8 + T cells (R = –0.132, P = 9.98e–03), and dendritic cells (R = –0.217, P = 2.12e–05).

3.5. Relationship between biomarkers of different immune cell subsets and Nuf2 gene expression

We analyzed the relationship between tumor infiltration immunocytes and Nuf2 through GC cells immunization biomarker gene expression. Different kinds immunocytes in GC tissues were analyzed by TIMER and GEPIA database, including B cells, T cells, and their respective subpopulations (adjuvant T cells 17 [Th17], helper T cells 2 [Th2], adjuvant T-cell 1 [Th1], exhausted T cells, follicular helper T cells [Tfh], regulatory T cells [Tregs], dendritic cells, monocytes, CD4 + T cells, CD8 + T cells, natural kill cells [NKS], neutrophil, M1 macrophage, M2 macrophages, and tumor-related macrophages [TAMS]).

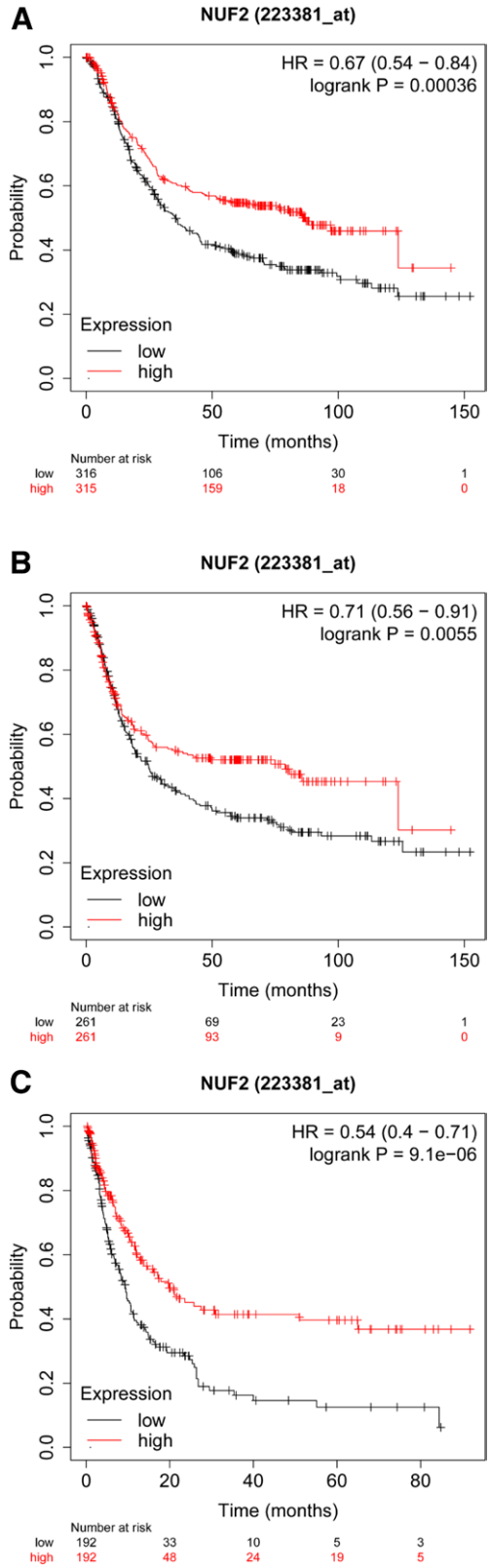

Figure 2. Relationship between Nuf2 expression and prognostic survival in GC patients via Kaplan–Meier plotter analysis. (A) Overall survival, n = 631; (B) First progression survival, n = 522; (C) Postprogression survival, n = 384.

The results showed that the expression of Nuf2 in GC and the expression of most biomarkers in immune cells have significant negative correlation (Table 2).

Table 1

Nuf2 expression in GC with clinicopathological factors by Kaplan–Meier plotter.

		Overall survival			First progression			Postprogression survival		
Characteristics		***N***	**Hazard ratio**	***P* value**	**N**	**Hazard ratio**	***P* value**	***N***	**Hazard ratio**	***P* value**
Gender	Female	187	0.53 (0.34–0.83)	.0043	179	0.61 (0.39–0.94)	.023	127	0.31 (0.18–0.53)	6.60E-06
	Male	349	0.69 (0.52–0.93)	.015	341	0.86 (0.64–1.15)	.3	256	0.65 (0.47–0.91)	.012
Her2 status	Negative	429	0.58 (0.44–0.76)	6.5E-05	356	0.67 (0.5–0.9)	.0076	283	0.58 (0.42–0.82)	.0016
	Positive	202	0.94 (0.65–1.37)	.76	166	0.85 (0.56–1.28)	.43	101	0.65 (0.4–1.07)	.087
Lauren classification	Instestinal	269	0.79 (0.55–1.14)	.2	263	0.87 (0.61–1.23)	.43	192	0.68 (0.45–1.03)	.064
	Diffuse	240	0.59 (0.42–0.84)	.0029	231	0.7 (0.49–0.99)	.041	176	0.62 (0.42–0.91)	.013
	Mixed	29	0.75 (0.25–2.23)	.6	28	1.78 (0.66–4.79)	.25	/	/	/
N status	N0	74	0.38(0.15–0.99)	.041	72	0.39 (0.15–1)	.042	41	0.33 (0.09–1.22)	.081
	N1	422	0.64 (0.49–0.84)	.0011	423	0.7 (0.55–0.91)	.0069	337	0.56 (0.42–0.75)	8.40E-05
M status	M0	444	0.63 (0.47–0.83)	.0012	443	0.69 (0.53–0.9)	.0069	342	0.55 (0.41–0.75)	.00013
	M1	56	0.51 (0.28–0.92)	.02	56	0.79 (0.44–1.44)	.44	36	0.4 (0.18–0.88)	.018

Bold values indicate $P < .05$. $P < .05$ denotes significance.

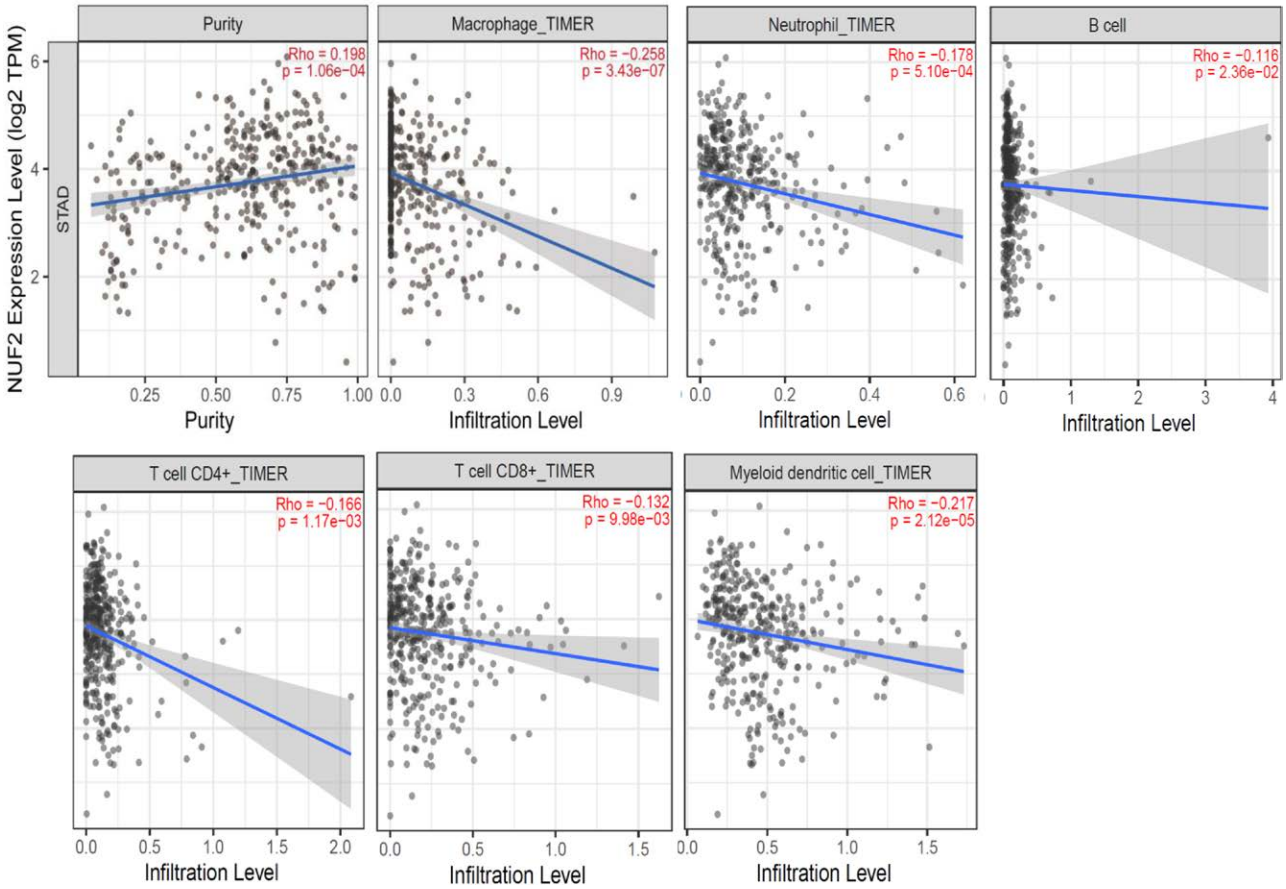

Figure 3. The analysis of immune cell infiltration for Nuf2 expression in GC tissues via TIMER. GC = gastric cancer.

By comparing the analysis results of TIMER and GEPIA, the results of the 2 databases are mostly consistent. CD8+T-cell biomarkers (CD8A), T-cell (general) biomarkers (CD2, CD3E, CD3D), B-cell biomarkers (CD19, CD79A), monocyte biomarkers (CD86, CD115), TAM biomarkers (CCL2, CD68, IL10), M2 macrophage biomarkers (CD163, VSIG4, MS4A4A), neutrophil biomarkers (CD11b, CCR7), dendritic cell biomarkers (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA-1, BDCA-4, CD11c), Th1 biomarkers (STAT4,STAT1), Th2 marker (GATA3, STAT5A), TFH marker (BCL6), and Tregs marker (transforming growth factor-β) all showed consistent and significant negative correlation in the results of the 2 data.

4. Discussion

Upper digestive tract cancer is a highly fatal malignant tumor. According to Global Cancer Statistics 2020, the number of new cases and deaths of GC in 2020 is about 1,089,103 and 768,793, respectively, ranking fifth and fourth among all cancer cases.[16] With the improvement of dietary structure and the progress of comprehensive treatment based on surgical treatment and other factors, the incidence and mortality of GC have been declining for decades, but GC is still the main type of cancer in the world and shows a younger trend, although surgery is the most important and effective method for radical cure of GC at present. However, in China, many patients with GC are already in

Table 2

Correlation analysis between Nuf2 and biomarker genes of immune cells in gastric cancer.

		Cor	FDR	Cor	FDR
CD8+ T cell	CD8A	−0.14	6.43E-03	−0.21	1.3E-05
	CD8B	0.021	0.0679	−0.035	0.48
T cell (general)	CD3D	−0.143	5.37E-03	−0.22	6.8E-06
	CD3E	−0.166	1.22E-03	−0.25	5.2E-07
	CD2	−0.118	0.022	−0.19	0.00013
B cell	CD19	−0.161	1.65E-03	−0.17	0.00066
	CD79A	−0.26	2.80E-07	−0.33	7.7e−12
Monocyte	CD86	−0.107	0.0378	−0.17	5e−04
	CD115	−0.258	3.37E-07	−0.26	5.9e−08
TAM	CCL2	−0.273	6.54E-08	−0.31	2.5e−10
	CD68	−0.121	0.0183	−0.14	0.0034
	IL10	−0.108	0.0348	−0.17	0.00063
M1 Macrophage	iNOS	0.063	0.219	0.11	0.027
	IRF5	−0.139	6.88E-03	−0.089	0.072
	COX2	−0.026	0.614	−0.072	0.15
M2 Macrophage	CD163	−0.142	5.45E-03	−0.2	3.2e−05
	VSIG4	−0.222	1.24E-05	−0.23	1.6e−06
	MS4A4A	−0.225	9.33E-06	−0.26	1.8e−07
Neutrophils	CD66b	0.149	3.74E-03	0.085	0.088
	CD11b	−0.198	1.00E-04	−0.22	1.1e−05
	CCR7	−0.283	2.05E-08	−0.35	3.9e−13
Natural killer cell	KIR2DL1	0.026	0.618	−0.066	0.19
	KIR2DL3	0.061	0.24	−0.054	0.28
	KIR2DL4	0.11	0.0316	0.025	0.61
	KIR3DL1	−0.024	0.647	−0.11	0.033
	KIR3DL2	0.018	0.724	−0.083	0.096
	KIR3DL3	0.049	0.342	0.033	0.51
	KIR2DS4	−0.011	0.831	−0.044	0.38
Dendritic cell	HLA-DPB1	−0.243	1.67E-06	−0.29	1.4e−09
	HLA-DQB1	−0.167	1.1E-03	−0.22	6.5e−06
	HLA-DRA	−0.153	2.77E-03	−0.2	6.5e−05
	HLA-DPA1	−0.2	8.7E-05	−0.16	0.0012
	BDCA-1	−0.377	3.10E-14	−0.29	1.8e−09
	BDCA-4	−0.208	4.44E-05	−0.1	0.04
	CD11c	−0.089	0.0833	−0.13	0.0071
Th1	T-bet	−0.097	0.0601	−0.15	0.0031
	STAT4	−0.145	4.82E-03	−0.12	0.017
	STAT1	0.218	1.79E-05	0.21	2.6e−05
	IFN-*g*	0.163	1.49E-03	0.0098	0.84
	TNF-*a*	0.006	0.906	−0.063	0.21
Th2	GATA3	−0.196	1.26E-04	−0.15	0.0021
	STAT6	−0.099	0.053	0.012	0.81
	STAT5A	−0.122	0.017	−0.12	0.016
	IL13	0.026	0.618	−0.099	0.046
Tfh	BCL6	−0.282	2.26E-08	−0.1	0.036
	IL21	0.121	0.0186	−0.02	0.69
Th17	STAT3	−0.113	0.0276	−0.042	0.4
	IL17A	0.171	8.01E-4	0.045	0.36
Treg	FOXP3	−0.014	0.782	−0.09	0.068
	CCR8	−0.043	0.407	−0.079	0.11
	STAT5B	−0.117	0.0227	−0.073	0.14
	TGF*b*	−0.237	3.13E−06	−0.18	0.00031
T cell exhaustion	PD-1	−0.014	0.782	0.0023	0.96
	CTLA4	0.093	0.0698	0.15	0.002
	LAG3	−0.023	0.658	−0.053	0.28
	TIM-3	−0.084	0.102	−0.11	0.033
	GZMB	0.113	0.0274	−0.02	0.69
	TOX	−0.258	3.4E-7	0.0044	0.93
	TIGIT	−0.06	0.241	−0.14	0.0035

Bold values indicate $P < .05$.

the middle and late stages when they are first diagnosed, invasion and metastasis, immune escape, and so on further reduce the long-term survival rate of patients with GC, so that the prognosis of patients with advanced GC is poor. In this study, through a series of bioinformatics analysis, combined with the open database, we analyzed the expression level of Nuf2 gene in GC and corresponding normal tissues, and the effects of Nuf2 expression on survival prognosis and immune cell infiltration in patients with GC. We found that the expression of Nuf2 in GC tissues was significantly higher than that in corresponding normal tissues, but an interesting phenomenon was that the OS time, FP, PPS of patients with high expression of Nuf2 were significantly better than those with low expression of Nuf2, indicating that Nuf2 may be a protective factor in patients with GC. This is different from previous studies, which suggested that Nuf2 is an oncogene in liver cancer and breast cancer.[17,18] Moreover, subgroup analysis showed that patients with high expression of Nuf2 had better prognosis in GC patients with Her2 negative, diffuse classification, and local lymph node positive and without distant metastasis, respectively.

While in patients with Her2-positive GC, other GC types, local lymph node negative and distant metastasis patients, different Nuf2 expression levels had no significant difference in prognosis. Zhai et al[18] demonstrated that the triple-negative breast cancer (TNBC) patients with higher NUF2 expression level had significantly reduced the survival. The results of several clinical trials after the ToGA study further verified the efficacy and safety of trastuzumab in the treatment of HER2 positive advanced GC.[19] Patients with HER2 positive gastric adenocarcinoma can use trastuzumab to improve prognosis, while the difference in the expression level of Nuf2 in HER2-negative patients indicates the potential to target Nuf2 gene. There is remain no research on the expression of Nuf2 in GC in HER2-negative, and its prognostic effects are required to be verified by further basic experiments. Gastric adenocarcinoma can be divided into intestinal and diffuse types. The prognosis of intestinal type GC is better, while the prognosis of diffuse type GC is worse, the cancer cells spread faster and it is more difficult to treat. The downregulation of tumor suppressor genes P27, P53, P1, and Rb, and the overexpression of oncogenes Myc and Bcl-2 in GC often indicates a poor prognosis.[20] The overexpression of matrix metalloproteinase-2 (MMP-2) and MMP-7 is related to lymph node metastasis, depth and extent of invasion, indicating a poor clinical outcome.[21] The expression level of homologous lost phosphatase-tensin gene (PTEN) on chromosome 10 was negatively correlated with invasion, metastasis, and Lauren classification of GC, suggesting that the suppression of PTEN expression indicates the malignant progression of GC.[22] Our results show that GC patients with high expression of Nuf2 have a good prognosis in diffuse GC, suggesting that Nuf2 may be a potential tumor suppressor gene in patients with gastric diffuse adenocarcinoma, and further study of molecular mechanism is needed.

Cell division-related gene Nuf2, as an important part of NDC80 complex, participates in kinetochore-microtubule adhesion and is involved in the important process of cell mitosis and tumorigenesis and development. Abnormal expression of Nuf2 in tumor tissue can induce mitotic cell abnormality and lead to cell death, which leads to tumor. In previous studies, it was suggested that the overexpression of Nuf2 was associated with poor prognosis.[17,18] But interestingly, in our study, there is a contradiction between the role of Nuf2 in patients with gastric adenocarcinoma and its role in other cancers, that is, high expression of Nuf2 shows a better prognosis. Therefore, further laboratory verification is needed to confirm the role of overexpression of Nuf2 in improving the prognosis of patients with gastric adenocarcinoma.

Several cytotoxic drugs are effective against GC, including fluoropyrimidines, platinum (cisplatin, oxaliplatin), taxanes (paclitaxel, docetaxel), and irinotecan. However, advances in molecular targeted therapy and immunotherapy have significantly changed first-line therapy in addition to cytotoxic chemotherapy alone. At present, it is believed that all patients with GC should evaluate the existence of mismatch repair defect (dMMR) microsatellite high instability (MSI-H) and programmed cell death ligand 1 (PD-L1) overexpression.[23,24] The rapid development

of immunotherapy brings a glimmer of light to the treatment of GC. In this study, it was found that there was a significant negative correlation between Nuf2 and a variety of immune cells, suggesting that Nuf2-mediated gastric carcinogenesis may mobilize the activity of these immune cells and make them play an antitumor role. In this study, we found that the expression of Nuf2 was significantly related to the biomarker genes of T cells, B cells, monocytes, TAM cells, M2 macrophages, dendritic cells, and other immune cells. These findings may contribute to the development of new immunotherapy for GC patients whose existing immunosuppressive checkpoints (such as PD-1) inhibitors are ineffective. However, the analysis results of Table 2 show that Nuf2 is only related to some biomarkers of immune cells, indicating that there is a certain specificity, which also provides a certain basis for future immunotherapy.

The advantage of this study is to use the sequencing data of GC in a variety of databases, including a large number of samples, and the clinical data are relatively complete. However, it needs to be emphasized that although bioinformatics analysis can comprehensively and quickly mine potential data and functional biomolecules, there is the possibility of false-positive results, which need to be further verified by cytological and molecular biology experiments. Therefore, the purpose of our work is to provide a fast and simple method for screening functional genes and to point out the direction for future research.

Author contributions

Conceptualization: Xin Xu and Lv'rong.
Ge; Formal analysis: :Lv' rong Ge and Qing Sun
Writing – original draft: Lv'rong Ge and Lian Xia.
Writing – review and editing: Xin Xu and Lv'rong Ge.

References

[1] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.

[2] Zhu AL, Sonnenberg A. Is gastric cancer again rising? J Clin Gastroenterol. 2012;46:804–6.

[3] Wroblewski LE, Peek RM, Wilson KTJCmr, et al. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010;23:713–39.

[4] Bertuccio P, Chatenoud L, Levi F, et al. Recent patterns in gastric cancer: a global overview. Int J Cancer. 2009;125:666–73.

[5] Hu P, Chen X, Sun J, et al. siRNA-mediated knockdown against nuf2 suppresses pancreatic cancer proliferation in vitro and in vivo. Biosci Rep. 2015;35:e00170.

[6] Obara W, Sato F, Takeda K, et al. Phase I clinical trial of cell division associated 1 (CDCA1) peptide vaccination for castration resistant prostate cancer. Cancer Sci. 2017;108:1452–7.

[7] Zhang T, Zhou Y, Qi ST, et al. Nuf2 is required for chromosome segregation during mouse oocyte meiotic maturation. Cell Cycle. 2015;14:2701–10.

[8] Harao M, Hirata S, Irie A, et al. Hla-a2-restricted CTL epitopes of a novel lung cancer-associated cancer testis antigen, cell division cycle associated 1, can induce tumor-reactive CTL. Int J Cancer. 2008;123:2616–25.

[9] Hayama S, Daigo Y, Kato T, et al. Activation of CDCA1-KNTC2, members of centromere protein complex, involved in pulmonary carcinogenesis. Cancer Res. 2006;66:10339–48.

[10] Sethi G, Pathak HB, Zhang H, et al. An RNA interference lethality screen of the human druggable genome to identify molecular vulnerabilities in epithelial ovarian cancer. PLoS One. 2012;7:e47086e47086.

[11] Rhodes DR, Kalyana-Sundaram S, Mahavisno V, et al. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia (New York, NY). 2007;9:166–80.

[12] Li T, Fu J, Zeng Z, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48:W509–14.

[13] Li T, Fan J, Wang B, et al. Timer: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.

[14] Nagy A, Munkacsy G, Gyorffy B. Pancancer survival analysis of cancer hallmark genes. Sci Rep. 2021;11:6047.

[15] Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45:W98–W102.

[16] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-Cancer J Clin. 2021;71:209–49.

[17] Xie X, Jiang S, Li X. Nuf2 is a prognostic-related biomarker and correlated with immune infiltrates in hepatocellular carcinoma. Front Oncol. 2021;11:621373.

[18] Zhai X, Yang Z, Liu X, et al. Identification of NUF2 and FAM83D as potential biomarkers in triple-negative breast cancer. PeerJ. 2020;8:e9975.

[19] Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687–97.

[20] Marin JJ, Al-Abdulla R, Lozano E, et al. Mechanisms of resistance to chemotherapy in gastric cancer. Anticancer Agents Med Chem. 2016;16:318–34.

[21] Kubben F, Sier C, Van Duijn W, et al. Matrix metalloproteinase-2 is a consistent prognostic factor in gastric cancer. Br J Cancer. 2006;94:1035–40.

[22] Zhang LL, Liu J, Lei S, et al. PTEN inhibits the invasion and metastasis of gastric cancer via downregulation of FAK expression. Cell Signal. 2014;26:1011–20.

[23] Boku N, Ryu MH, Kato K, et al. Safety and efficacy of nivolumab in combination with S-1/capecitabine plus oxaliplatin in patients with previously untreated, unresectable, advanced, or recurrent gastric/gastroesophageal junction cancer: interim results of a randomized, phase II trial (ATTRACTION-4). Ann Oncol. 2019;30:250–8.

[24] Shitara K, Van Cutsem E, Bang YJ, et al. Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with first-line, advanced gastric cancer: the keynote-062 phase 3 randomized clinical trial. JAMA Oncol. 2020;6:1571–80.