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Article

Prognostic Value and Biological Significance of GUCY1A2 in Gastric Cancer: A Bioinformatics Analysis Base on TCGA Database

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Abstract: ***Background.*** Guanylate cyclase 1 soluble subunit alpha 2 (*sGCα2*), also known as *GUCY1A2*, was reported to be upregulated and promoted tumorigenesis in cervical cancer. But whether *GUCY1A2* was abnormally expressed and its prognostic value in gastric cancer was unknown. The current study aimed to find out the prognostic value of *GUCY1A2* in gastric cancer by analyzing data from The Cancer Genome Atlas (TCGA) database. ***Methods.*** Wilcoxon signed-rank test, cox regression analysis and multivariant analysis were used to analyze the relationship between clinical characteristic and *GUCY1A2* expression level. Kaplan-Meier method was used to analyze the association of *GUCY1A2* and overall survival. Gene set enrichment analysis (GSEA) was used to identify *GUCY1A2*-related signaling pathway. ***Results.*** Compared to normal tissue, expression of *GUCY1A2* was significantly increased in gastric cancer (p<0.001). Increased *GUCY1A2* was associated with advanced T stage (p=0.012) and poor survival (p=0.022). Univariate analysis showed that high *GUCY1A2* expression was associated with a poor overall survival (HR:1.44, 95% confidence interval [CI]: 1.03-2.02, p=0.03). Multivariate analysis indicated that *GUCY1A3* remained an independent prognostic predictor of overall survival (HR:1.75, 95% confidence interval [CI]: 1.20-2.56, p=0.00). GSEA revealed that calcium signaling pathway, *MAPK* signaling pathway, *TGF-β* signaling pathway and *Wnt* signaling pathway were enriched in *GUCY1A2* high expression phenotype. ***Conclusions.*** *GUCY1A2* maybe a potential prognostic predictor of poor survival in gastric cancer. But it need to be further validated clinically.

Keywords: Gastric Cancer, *GUCY1A2*, Prognosis, Survival

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1. Introduction

Gastric cancer is one of the most common malignant tumors in the world, with the fifth highest incidence rate and the third highest mortality rate [1]. The incidence rate of gastric cancer shows geographical differences. Gastric cancer most frequently occurred in East Asia, South America and Eastern Europe and was less common seen in Western Europe, Australia and North America [2]. More than 95% of gastric cancers are classified as adenocarcinomas [3]. Helicobacter pylori infection is one of the most important causes of gastric cancer [4]. Other risk factors include smoking, alcohol consumption, high salt intake, et al [5-7]. The only curative therapy for gastric cancer is surgical resection [8]. However, about 50% of patients presents with advanced diseases at diagnosis and about 40-60% of patients relapsed after surgical resection [9]. The standard treatment of these patients is systemic chemotherapy and target therapy [10]. However, prognosis of metastasis gastric cancer is poor, with the 5-year overall survival (OS) rate of 5-20% [11]. Searching

for new prognostic biomarkers and treatment targets may help to improve the prognosis of gastric cancer.

The cyclic GMP (cGMP)/protein Kinase G (PKG) pathway participates in the endogenous apoptotic process and relates to the development of some cancers, such as breast cancer and colon cancer [12-14]. cGMP, which is one of the most important intracellular signaling molecules in mammal cells, is generated from GTP by two classes of guanylate cyclases (GCs): soluble GC (sGC) and particulate GC (pGC) [15]. Guanylate cyclase 1 soluble subunit alpha 1 (sGCα1) and alpha 2 (sGCα2) are subunits of sGCs. It is reported that sGCα1 is increased in prostate cancer and overexpression of sGCα1 is related to chemoresistance [16]. Another research showes that sGCα1 promotes cell proliferation, migration and survival of cervical cancer cells [17]. Inhibition of basal activity of sGC/cGMP pathway induces p53-dependent apoptosis in ovarian cancer cells. Guanylate cyclase soluble subunit alpha-2, also known as GUCY1A2, is found to be upregulated in cervical cancer and correlated with superficial tumor growth [18]. However, the expression level and prognostic value of GUCY1A2 in gastric cancer remains unknown.

Thus, the current study aimed to evaluate the expression pattern and prognostic value of GUCY1A2 in gastric cancer by bioinformatics analysis. In addition, we performed the gene set enrichment analysis (GSEA) to study the biological pathway related to the GUCY1A2 regulation. The data used in this study was obtained from TCGA database (https://portal.gdc.cancer.gov/).

2. Materials and methods

2.1. TCGA data download and bioinformatic analysis

The gene expression data and corresponding clinical data of gastric cancer (project ID: TCGA-STAD) were downloaded from TCGA database (https://portal.gdc.cancer.gov/). Expression data of GUCY1A2 was extracted. Then the expression difference of GUCY1A2 between normal and tumor samples were compared. Clinical information was extracted and patients with incomplete clinical information were excluded. Finally, there were 349 patients were included into further analysis. The media expression value of GUCY1A2 were calculated. Patients with GUCY1A2 expression level higher than media value were distributed to the high expression group and others were distributed to the low expression group. Overall survival in high expression and low expression groups were compared. Univariate and multivariate cox regression analysis were used to analysis the influence of clinical characteristics on the survival.

2.2. Gene set enrichment analysis

Software GSEA_4.1.0 was used to perform gene set enrichment analysis (GSEA). The expression matrix file and phenotype file were prepared before GSEA. GUCY1A2 expression level was used as a phenotype label. Gene set permutations number was set to 1000. Then the expression matrix file and phenotype file were imported and GSEA was performed.

2.3. Statistical analysis

Statistical analyses were conducted by R software (R x64 3.6.2). The comparison of GUCY1A2 expression between normal and tumor sample was analyzed by Wilcoxon signed-rank test. Clinical characteristics associated to survival was analyzed by Cox regression and Kaplan-Meier method. To further compare the effect of GUCY1A2 expression on overall survival along with other clinical factors, multivariate Cox analysis was performed.

3. Results

3.1. Patient characteristics

Totally 349 cases with clinical data were downloaded from TCGA in January 2021. 136 (39.0%) cases were male. 47 (13.5%) patients were with stage I disease, 110 (31.5%) patients were with stage II disease, 155 (44.4%) patients were with stage III disease and 37 (10.6%) patients were with stage IV disease. T1, T2, T3, T4 diseases were found in 17 (4.9%), 72 (20.6%), 168 (48.1%), 92 (26.4%) patients, respectively. 67.9% of the patients had lymph node invasion. Of which, 91 cases were N1 disease, 75 cases were N2 disease, 71 cases were N3 disease (Figure S1). 24(6.9%) patients had distant metastases. Clinical characteristics of patients were shown in Table 1.

Table 1. Patient characteristics of gastric cancer

Characteristic		n(total=349)	%
Age	≤65	160	45.8
	＞65	189	54.2
Gender	male	136	39.0
	female	213	61.0
Grade	G1	6	1.7
	G2	120	34.4
	G3	223	63.9
Clinical stage	I	47	13.5
	II	110	31.5
	III	155	44.4
	IV	37	10.6
T stage	1	17	4.9
	2	72	20.6
	3	168	48.1
	4	92	26.4
N stage	0	112	32.1
	1	91	26.1
	2	75	21.5
	3	71	20.3
M stage	0	325	93.1
	1	24	6.9

3.2. GUCY1A2 expression was increased in gastric carcinoma patients

Data of 349 gastric carcinoma samples and 30 normal samples with GUCY1A2 expression information were downloaded from TCGA database in January 2021. GUCY1A2 expression level were compared between normal samples and tumors samples. In comparison to normal samples, GUCY1A2 expression was significantly increased in tumor samples (Figure 1A). We also compared GUCY1A2 expression level between normal and tumor samples from the same patient. Result showed that GUCY1A2 was up-regulated in tumor samples (Figure 1B).

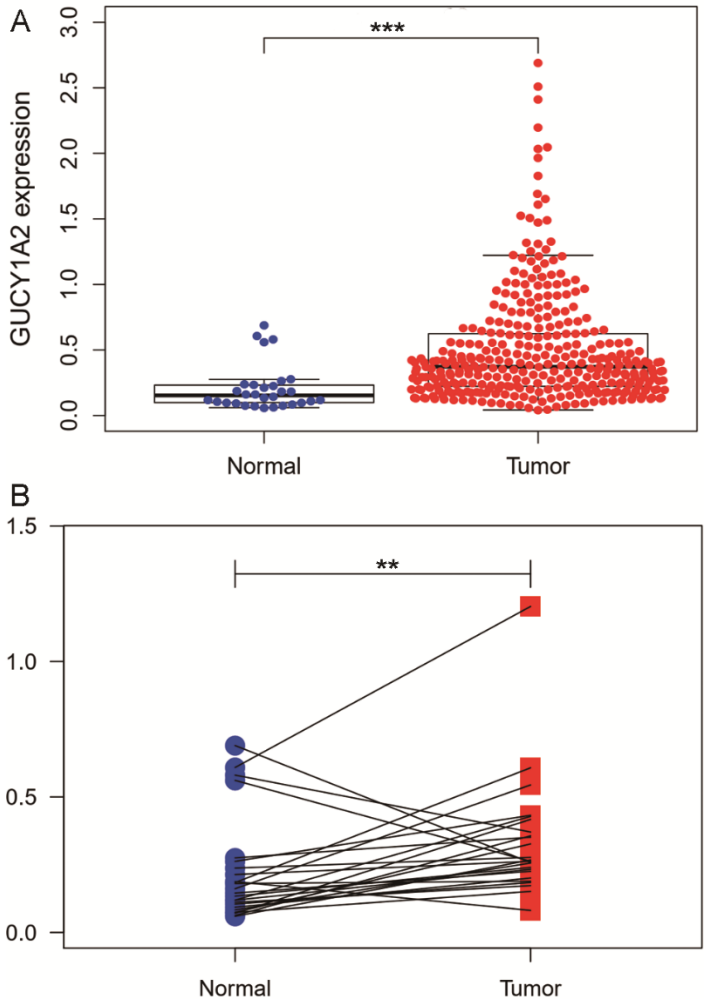

Figure 1. (A) GUCY1A2 expression in gastric cancer was significantly higher than normal samples (B) GUCY1A2 expression in cancer sample was also higher than normal sample from the same patient. **p<0.01, ***p<0.001

3.3. Increased GUCY1A2 was associated with poor overall survival

Patients with complete survival information and GUCY1A2 expression information were included in survival analysis. As shown in Figure 2A, patients with high GUCY1A2 expression showed a worse prognosis than patients with low GUCY1A2 expression. We also analyzed the association between GUCY1A2 expression and other clinical characteristics. Results showed that expression level of GUCY1A2 was correlated to histological grade and T stage. Patients who with higher histological grade and T stage had higher GUCY1A2 level. But GUCY1A2 expression was independent of age, gender, clinical stage, lymph node invasion or distant metastasis (Figure 2B-2H).

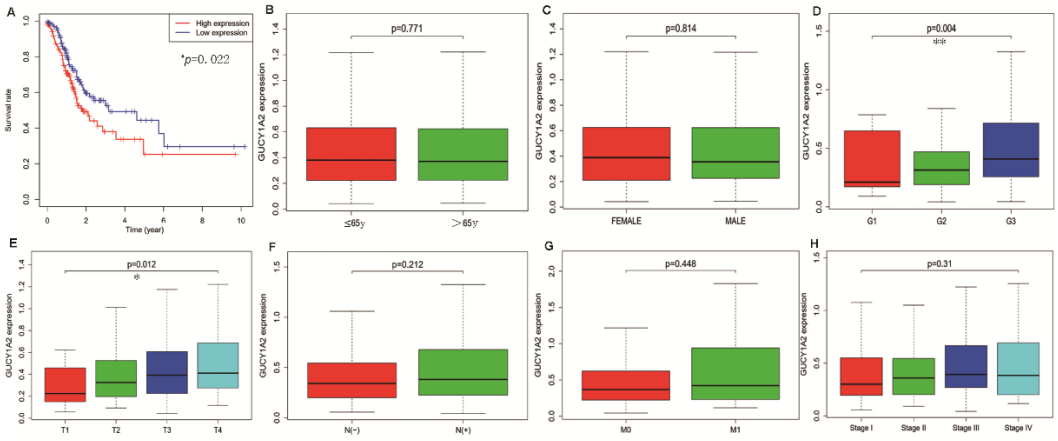

Figure 2. The correlation of GUCY1A2 expression with survival and other clinical characteristics. (A) Patients with increased GUCY1A2 expression showed worse survival. (B) Correlation of GUCY1A2 expression and age. (C) Correlation of GUCY1A2 expression and gender. (D) Correlation of GUCY1A2 expression and histological grade. (E) Correlation of GUCY1A2 expression and T stage. (F) Correlation of GUCY1A2 expression and lymph nodes metastasis. (G) Correlation of GUCY1A2 expression and distant metastasis. (H) Correlation of GUCY1A2 expression and clinical stage. *p<0.05, **p<0.01, ***p<0.001

3.4. GUCY1A2 was an independent prognostic factor in gastric carcinoma

Univariate cox regression analysis showed that age, clinical stage, distant metastasis and high GUCY1A2 expression level was correlated significantly with poor survival. HR was1.44 and 95% confidence intervals was1.03-2.02 (p=0.03) for GUCY1A2(Table 2a). Age, gender, clinical stage and increased GUCY1A2 expression level remained associated with survival at multivariate analysis (Table 2b and Figure 3).

Table 2. Association of clinical characteristics and survival by univariate cox regression and Multivariate analysis.

Characteristic	HR(95%CI)	p-value
a. Univariate cox regression		
Age	1.03(1.01-1.05)	0.004*
Gender	1.50(0.98-2.30)	0.062
Grade	1.25(0.85-1.84)	0.252
Clinical stage	1.51(1.20-1.91)	0.001*
T stage	1.28(1.00-1.63)	0.050
M stage	2.07(1.07-3.98)	0.030*
N stage	1.54(0.97-2.45)	0.070
GUCY1A2	1.44(1.03-2.02)	0.034*
b. Multivariate analysis		
Age	1.05(1.02-1.07)	0.000*
Gender	1.57(1.01-2.44)	0.045*
Grade	1.33(0.89-1.99)	0.167
Clinical stage	1.71(1.09-2.69)	0.019*
T stage	1.03(0.73-1.45)	0.086
M stage	1.83(0.80-4.16)	0.150*
N stage	0.69(0.34-1.38)	0.292
GUCY1A2	1.76(1.20-2.56)	0.004*

HR: Hazard Ratio, CI: confident interval; *, p<0.05

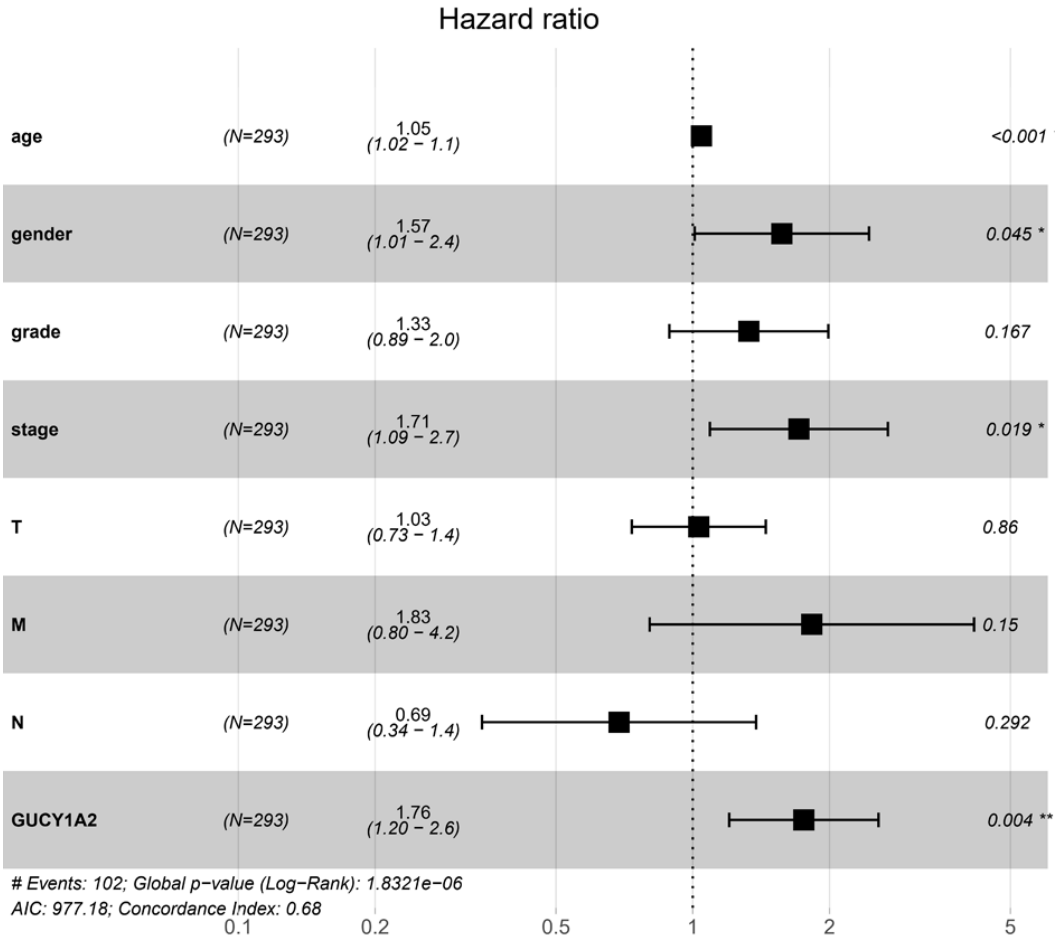

Figure 3. Multivariate analysis showed that age, gender, clinical stage and high expression of GUCY1A2 were independent predictors of poor survival.

3.5. GUCY1A2 related signaling pathways identified by GSEA

We performed GSEA to find out signaling pathways which were differentially activated between low GUCY1A2 expression and high GUCY1A2 expression data sets in gastric carcinoma. Differences in enrichment of KEGG pathways (c2.cp.kegg.v7.2.symbols) were analyzed. Pathways of adherens junction, calcium signaling pathway, cell adhesion molecules cams, ECM receptor interaction, focal adhesion, MAPK signaling pathway, TGF-β signaling pathway, Wnt signaling pathway were enriched in GUCY1A2 high expression group (Figure 4).

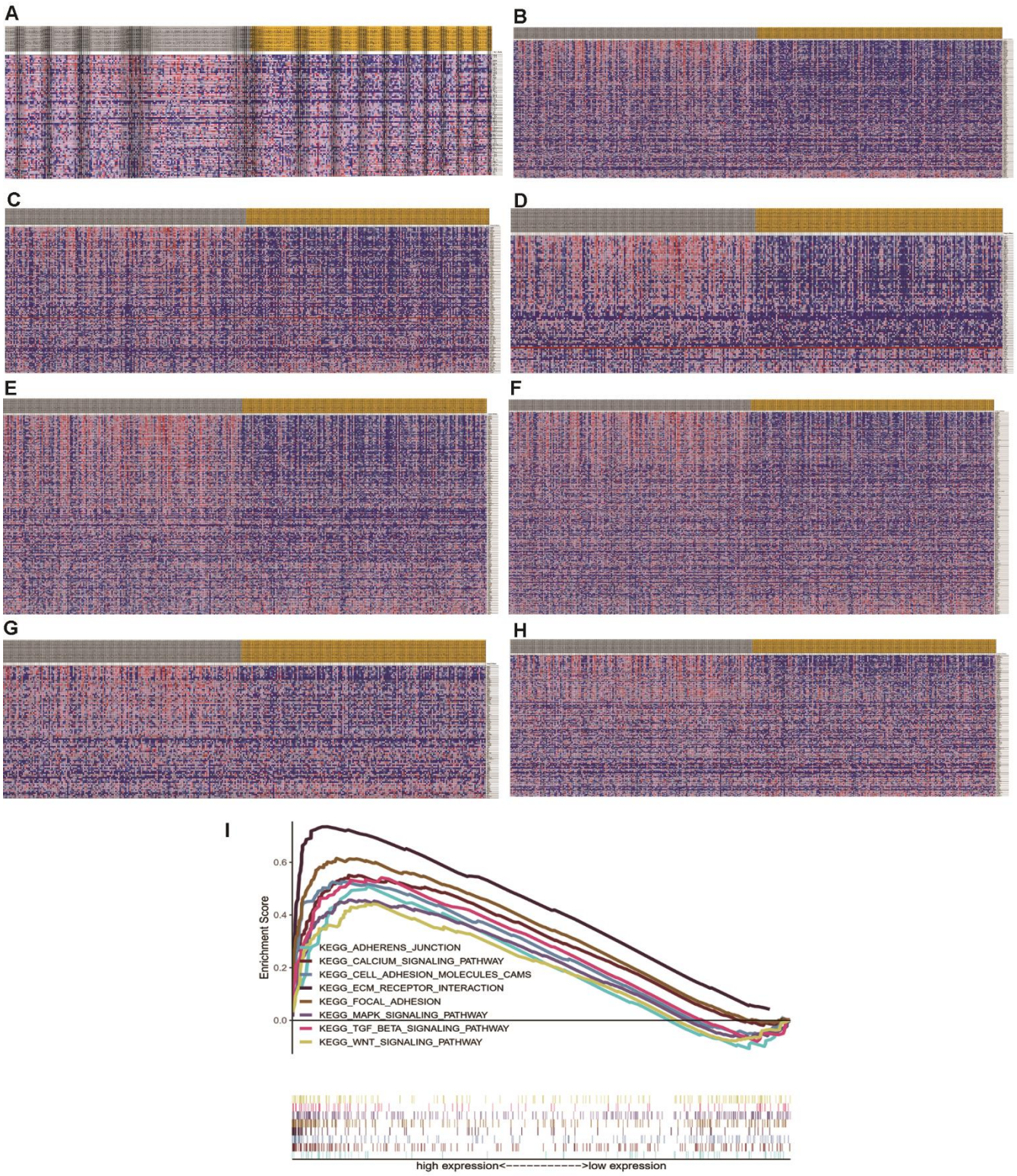

Figure 4. Results of gene set enrichment analysis showed that adherens junction, calcium signaling pathway, cell adhesion molecules cams, ECM receptor interaction, focal adhesion, MAPK signaling pathway, TGF-β signaling pathway, Wnt signaling pathway were enriched in GUCY1A2 high expression group. (A) KEGG adherens junction pathway. (B) KEGG calcium signaling pathway. (C)KEGG cell adhesion molecules cams. (D) KEGG ECM receptor interaction pathway. (E)KEGG focal adhesion pathway. (F)KEGG MAPK signaling pathway. (G)TGF-β signaling pathway. (H)Wnt signaling pathway. (I) Gene set enrichment analysis result of the above pathways.

4. Discussion

Soluble guanylate cyclase (sGC) is a catalytic enzyme catalyzing the conversion of guanosine 5′-triphosphate (GTP) into cyclic guanosine 3′,5′-monophosphate (cGMP). sGC is one of the main receptors of nitric oxide (NO). When sGC binds to NO, the catalytic activity was increased by 100- to 200-folds. Thus, sGC plays an important role in the NO-cGMP pathway [19]. It has been shown that NO/sGC/cGMP pathway plays an important role in tumor development. Increased cGMP leads to increased protein kinase G (PKG) activity and promotes prostate cancer cell proliferation [20]. Inhibition of NO/cGMP/PKG pathway results in suppression of migration and invasion in breast cancer cells [21].

sGC is a 150kDa heterodimer consisting of two subunits, subunit α and subunit β [22]. Both of the subunits are required for catalytic activity [23]. There are two isoforms of both subunits，α1, α2 and β1，β2. α1/β1 is the most abundant form. But there is indistinguishable activity between the α1/β1 and α2/β1 heterodimers [24]. It has been shown that sGC subunit α1, also known as GUCY1A2, is increased in some type of cancers and promotes cell proliferation, migration and survival of cancer cells [16-18]. But whether GUCY1A2 was abnormally expressed and the prognostic value in gastric cancer was unknown.

In the current study, we found that GUCY1A2 expression was increased in gastric cancer in comparison to normal tissues. Increased GUCY1A2 expression was associated with advanced T stage and poor prognosis in gastric cancer. GSEA indicated that adherens junction, calcium signaling pathway, cell adhesion molecules cams, ECM receptor interaction, focal adhesion, MAPK signaling pathway, TGF-β signaling pathway, Wnt signaling pathway was enriched in GUCY1A2 high expression phenotype. Results suggested that high level of GUCY1A2 may serve as a predictive biomarker of poor prognosis in gastric cancer.

Some research has indicated that upregulation of the calcium signaling pathway, Wnt signaling pathway, MAPK signaling pathway and TGF-β signaling pathway were associated with tumor development and progression [25-28]. A recent study showed that increased cGMP resulted in increased tumor cell stemness and promoted metastasis in breast cancer by activating the cGMP-dependent PKG and MAPK signaling pathway [29]. Activation of sGC and increased generation of cGMP also induced activation of TGF-β1_ENREF_30 [30]. However, little is known about how sGC subunit GUCY1A2 was related to the above signaling pathway and further exploration is needed.

Our current research showed that GUCY1A2 mays serve as a potential prognostic biomarker of poor survival in gastric cancer. Calcium signaling pathway, MAPK signaling pathway, TGF-β signaling pathway and Wnt signaling pathway maybe the critical pathway regulated by GUCY1A2 in gastric cancer. However, GUCY1A2 expression level in this research was mRNA expression rather than protein expression and using mRNA to predict protein expression was not perfect. Further study is needed to validate the results and prove the biological influence of GUCY1A2.

Supplementary material

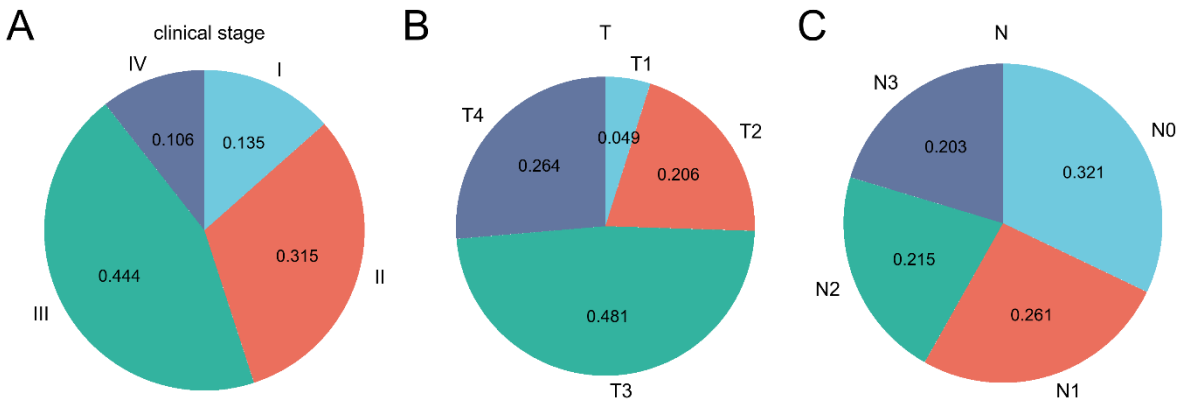

Figure S1. Proportion of patients with different clinical characteristics. (A) Clinical stage. (B) T stage. (C) N stage.

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Conflict of Interest Statement: None declared.

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