Prognostic value and biological significance of GUCY1A2 in gastric cancer: a bioinformatics analysis

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Research Article

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Abstract

Background: Guanylate cyclase 1 soluble subunit alpha 2 (sGC α 2), also known as GUCY1A2, was reported to be upregulated and promoted tumorigenesis in some cancers. But whether GUCY1A2 was abnormally expressed and the 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). **Methods:** Wilcoxon signed-rank test, cox regression analysis and multivariate 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 ($p=0.000$). Increased GUCY1A2 was associated with advance 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. Calcium signaling pathway, MAPK signaling pathway, TGF- β signaling pathway and Wnt signaling pathway may be the key signaling pathway regulated by GUCY1A2.

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. It is most frequently occurred in East Asia, South America and Eastern Europe and 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 the most important cause 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 in several types of cancers(12-14). cGMP, which is one of the most important intracellular

signaling molecule 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 showed that sGCα1 promoted cell proliferation, migration and survival of cervical cancer cells(17). Inhibition of basal activity of sGC/cGMP pathway induced 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 at evaluating the expression 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 GUCY1 regulation. The data used in this study was obtained from TCGA database (<https://portal.gdc.cancer.gov/>).

2. 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 cox regression and multivariate cox 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 group were compared. Cox regression and multivariate cox 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. 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 343 gastric carcinoma samples and 30 normal samples with GUCY1A2 expression information were downloaded from TCGA database in January 2021. GUCY1A2 expression level were compared in normal samples and tumors samples. In comparison to normal samples, GUCY1A2 expression was significantly increased in tumor samples (Fig. 1A and 1B).

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 Fig. 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, 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 (Fig. 2B-2H).

3.4 GUCY1A2 was an independent prognostic factor in gastric carcinoma

Univariate cox regression analysis showed that age, stage, distant metastasis and high GUCY1A2 expression level was correlated significantly with poor survival. HR was 1.44 and 95% confidence intervals was 1.03-2.02 ($p=0.03$) (Tab. 2). Age, gender, clinical stage and increased GUCY1A2 expression level remained associated with survival at multivariate analysis (Tab. 3 and Fig. 3).

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

Characteristic	HR(95%CI)	p value
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*

HR: Hazard Ratio, CI: confident interval

Table 3. Association of clinical characteristics and survival by Multivariate analysis

Characteristic	HR(95%CI)	p value
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

3.5 GUCY1A2 related signaling pathways identified by GSEA

We performed GSEA to found out signaling pathways which are 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. The enriched pathways were selected according to the normalized enrichment score (NES) (Tab.4). 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 (Fig. 4).

Table 4. Gene set enriched in HIST1H1D high phenotype

Gene Set Name	NES	NOM p-val
KEGG_CALCIIUM_SIGNALING_PATHWAY	2.08	0.000
KEGG_FOCAL_ADHESION	2.06	0.002
KEGG_ECM_RECEPTOR_INTERACTION	2.23	0.000
KEGG_TGF_BETA_SIGNALING_PATHWAY	1.80	0.016
KEGG_MAPK_SIGNALING_PATHWAY	1.76	0.008
KEGG_ADHERENS_JUNCTION	1.68	0.032
KEGG_CELL_ADHESION_MOLECULES_CAMS	1.65	0.048
KEGG_WNT_SIGNALING_PATHWAY	1.59	0.038

NES: Normalized enrichment score; NOM: Nominal. Gene sets with NOM p-val<0.05 were considered as significant.

4. Discussion

Soluble guanylate cyclase (sGC) is a catalytic enzyme that 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 it 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 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— $\alpha 1$, $\alpha 2$ and $\beta 1$ — $\beta 2$. $\alpha 1/\beta 1$ is the most abundant form. But there is indistinguishable activity between the $\alpha 1/\beta 1$ and $\alpha 2/\beta 1$ heterodimers(24). It has been shown that sGC subunit $\alpha 1$ and $\alpha 2$, also known as GUCY1A1 and 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 advance 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- β 1(30). However, little is known about how sGC subunit GUCY1A2 was related to the above signaling pathway and further exploration is needed.

5. Conclusion

Our current research showed that GUCY1A2 may 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.

List Of Abbreviations

TCGA, The Cancer Genome Atlas

GSEA, gene set enrichment analysis

KEGG, Kyoto Encyclopedia of Genes and Genomes

GUCY1A2, guanylate cyclase soluble subunit alpha-2

sGC, soluble Guanylate cyclase

OS, overall survival

cGMP, cyclic guanosine monophosphate

PKG, protein kinase G

TGF- β , transforming growth factor β

MAPK, mitogen activated protein kinase

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analysed during the current study are available in the TCGA repository, <http://cancergenome.nih.gov/>.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SH, MW, MZ and XP had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis;

Study concept and design: XP,

Acquisition of data: SH, MW, MZ

Analysis and interpretation of data: SH and MW

Drafting of the manuscript: SH, MW, MZ, XP

Statistical analysis: SH and MW

Supervision: MZ and XP

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Figures

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. ** $p < 0.01$, *** $p < 0.001$

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. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Hazard ratio

Figure 3

Multivariate analysis showed that age, gender, clinical stage and high expression of GUCY1A2 was independent predictors of poor survival. *p<0.05, **p<0.01

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.