Original Article

Prognostic biomarkers based on *GUF1*, *EFTUD2* and *GSPT1* targets affecting migration of gastric cancer cells

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Background: Eukaryotic elongation factor 1 alpha 2 (eEF1A2) is a protein coding gene which is involved in tumor development and progression in several types of human cancer, but little is known about the function of eEF1A2 proteins in gastric cancer (GC). This study aimed to investigate the effects of *GUF1*, *EFTUD2* and *GSPT1* on the migration of GC cells.

Methods: The Oncomine and The Cancer Genome Atlas (TCGA) databases were used to evaluate the expression of *GUF1*, *EFTUD2*, *GSPT1* and *GSPT2* in GC and the association of eEF1A2 family with individual clinical characteristics. Kaplan-Meier (K-M) Plotter hinted the prognostic value of *GUF1*, *EFTUD2*, *GSPT1* and *GSPT2*. GSE62254 and GSE66222 datasets were used to validate the expression of *GUF1*, *EFTUD2*, *GSPT1*. AGS cell line and GES line were also used for validating the function of *GUF1*, *EFTUD2*, *GSPT1*. RNA interference (RNAi) of GUF1, EFTUD2 and GSPT1 had been used to query those genes expression pattern and dissect the proliferation and migration in GC cell lines.

Results: *GUF1*, *EFTUD2* and *GSPT1* were significantly up-regulated in GC cell lines. High expression of *GUF1*, *EFTUD2* and *GSPT1* was correlated with cell proliferation and migration induced in GC cells. *GUF1*, *EFTUD2* and *GSPT1* may be potential novel oncogenes that helps to maintain the survival of GC cells.

Conclusions: This study identified that high levels of *GUF1*, *EFTUD2* and *GSPT1* expression are predictive biomarkers for a poor prognosis in GC.

Keywords: Gastric cancer (GC); GUF1; EFTUD2; GSPT1; prognosis

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Introduction

The translational process of protein synthesis is considered to be an important clue for understanding oncogenesis (1). Alternatively, eukaryotic elongation factor 1 alpha (eEF1A) proteins may enhance tumorigenesis independent of the protein translation network. eEF1A proteins are known to associate with actin and tubulin, and ectopic eEF1A expression can decrease the length of actin filaments and tubulin microtubules (2,3). Perhaps, the ability of eEF1A to alter cell structure somehow contributes to a neoplastic phenotype. While it is unclear how eEF1A2 might control cell growth, the process of protein synthesis in eukaryotic cells is controlled by various translation factors, such as eukaryotic initiation factor (eIF), eukaryotic elongation factor (eEF), and eukaryotic release factor (eRF) that, respectively, regulate initiation, elongation, and

termination, eEF1A2 is a protein coding gene. eEF1A2 is associated with conditions such as mental retardation, autosomal dominant, and developmental and epileptic encephalopathy. The pathways associated with eEF1A2 include protein metabolism and viral mRNA translation. Results of eEF1A2 cDNA hybridization in the cancer profiling array reveals significant overexpression of the transcript for eEF1A2 among several different cancer samples, including breast (50 pairs), uterus (42 pairs), colon (34 pairs), stomach (28 pairs) (4). Further investigation is necessary to determine the precise role for eEF1A2 in controlling the development of gastric cancer (GC). To date, 18 *eEF1A2* genes have been identified in mammalian cells, which are numbered in the order of their discovery (*eEF1A1*, *HBS1L*, *GSPT1*, *GSPT2*, *EEF2*, *TUFM*, *EIF5B*, *EFL1*, *GFM1*, *EIF2S3*, *MTIF2*, *GTPBP1*, *GFM2*, *EIF2S3B*, *GTPBP2*, *EFTUD2*, *EEFSEC*, *GUF1*). In a comprehensive analysis, only GUF1, EFTUD2, and GSPT1 are shown to be associated with GC. Therefore, we undertook the investigation of the involvement of these genes in GC progression and prognosis. *GUF1*, *EFTUD2*, *GSPT1* regulate protein translation and play an important role in tumor (5-7). Minor sites of normal eEF1A2 expression have been found in several specialized human body parts (8), including breast acini (9), glucagon-producing islet cells in the pancreas, Purkinje cells of the cerebellum (10), and lung alveoli (11). Nevertheless, the roles of *GUF1*, *EFTUD2* and *GSPT1* have been less studied in GC.

GC is one of the most common malignancies, ranking fifth and fourth in incidence and mortality among all malignancies worldwide respectively (12). In most countries, early GC screening is not carried out effectively, often resulting in a diagnosis at an advanced stage (13,14). It is important to examine the molecular mechanism underlying GC development and explore specific and efficient diagnostic markers and therapeutic targets. GC is a multifactorial disease caused by genetic and epigenetic changes, including alteration in DNA methylation and aberrant expression of noncoding RNAs. The treatment of GC has advanced recently; however, its prognosis has not been significantly enhanced and the prognosis of different individuals varies greatly. In recent years, to better guide the clinical treatment of GC, researchers have been working hard to identify biological markers of GC that could enable effective screening of people for susceptibility to GC and predict the survival patient with GC. Such biological markers of tumors can be of immense benefit in early diagnosis, clinical staging, prognosis and treatment efficacy assessment of GC.

While there is research (15) established that the eEF1A2 is genetically amplified and overexpressed in ovarian tumors and has oncogenic properties, it is yet to be shown whether eEF1A2 expression can directly cause other cancers such as GC in animals. Furthermore, we are yet to understand the mechanism by which eEF1A2 promotes tumorigenesis. In the absence of mechanistic insight, there are still important issues that need to be addressed with regard to eEF1A2 and GC. Firstly, is eEF1A2 copy number or eEF1A2 protein expression an GC prognostic factor? Secondly, is eEF1A2 a suitable target for anticancer therapy? In this study, we examined the expression of *GUF1*, *EFTUD2*, *GSPT1* and GSPT2 from eEF1A2 in normal tissues and GC tissues, and various cell lines. Meanwhile, we also evaluated the prognostic value of *GUF1*, *EFTUD2* and *GSPT1* in GC based on The Cancer Genome Atlas (TCGA) dataset. Subsequently, we analyzed the association between the *GUF1*, *EFTUD2* and *GSPT1* expression levels and individual pathologic stage, clinical T stage and individual primary therapy outcome, and diagnostic capabilities. The Gene Expression Omnibus (GEO) databases were used to verify the expressions of *GUF1*, *EFTUD2* and *GSPT1* in GC. We also evaluated their role in the development of GC through bioinformatics analysis and experimental verification. Our study provides information to help in the early diagnosis of GC. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-125/rc).

Highlight box

Key findings

- Genes (*GUF1*, *EFTUD2* and *GSPT1*) was selected to dissect the proliferation and migration in gastric cancer (GC) cell lines.

What is known and what is new?

- *GUF1*, *EFTUD2*, and *GSPT1* regulate protein translation and play an important role in tumor
- This study identified that high levels of *GUF1*, *EFTUD2* and *GSPT1* expression are predictive biomarkers for a poor prognosis in GC.

What is the implication, and what should change now?

- *GUF1*, *EFTUD2* and *GSPT1* have diverse functions and complex regulatory mechanisms in cancer. The mechanism in tumors has not been studied to a great degree yet. However, there is no doubt that *GUF1*, *EFTUD2* and *GSPT1* play an important role in GC tumorigenesis and development and may be potential targets for clinical diagnosis and treatment of GC.

Methods

Databases

We investigated the expression level of GUF1, EFTUD2 and GSPT1 in various types of normal tissues and tumor in the integrated datasets combining TCGA (https://portal.gdc.cancer.gov) with the GTEx (Genotype-Tissue Expression) database (https://www.gtexportal.org/ home/-index.html). TCGA are open-ended and public and do not need the approval of a local ethics committee. We obtained the profiles of RNA expression (RNA-Seq2 level 3 data; format: TPM; platform: Illumina HiSeq 2000) and clinical samples of GUF1, EFTUD2 and GSPT1 patients from the TCGA database. TCGA included 375 GC samples and 32 normal gastric tissue samples, which contain general information, clinicopathological details, and prognostic information. The GEO databases were filtered to remove missing and duplicated results by stringr and dplyr, the difference analysis by package limma, and whole data transformed by $\log_2$ (TPM +1) using R package of "ggstatsplot" in an R environment (R version: 3.6.1).

For analysis, we chose the following detailed databases: Gene Cards, Oncomine (https://www.oncomine. org/ resource/ login. html, an online cancer microarray database), TCGA (https://portal.gdc.cancer.gov) with the GTEx database (https://www.gtexportal.org/home/-index.html). Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia2.cancer-pku.cn/#index), and Kaplan-Meier Plotter (www.kmplot.com). The Human Protein Atlas (https://www.proteinatlas.org/humanproteome/pathology), and the GEO databases (GSE66229, GSE62254). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Expression differential analysis

Oncomine gene expression array dataset was used to analyze the transcription levels of eEF1A2 in different cancers. The mRNA level of eEF1A2 in clinical cancer specimens were compared with those in controls, using Student's *t*-test to generate a P value. The cutoffs of P value and fold change were 0.01 and 0.5.

Kaplan-Meier plot analysis

The prognostic value of the signal transducer and the activator of transcription (STAT) mRNA expression was evaluated using an online database, Kaplan-Meier Plotter contained gene expression data and survival information of GC patients (http://www.kmplot.com/analysis/index.php?p=service & cancer=gastric). To analyze the overall survival (OS), progression-free survival (FP), and post-progression survival (PPS) of patients with GC, the samples were split into two groups by median expression (high versus low expression) and assessed by a Kaplan-Meier survival plot, with a hazard ratio (HR) of 95% confidence intervals (CIs) and log rank P value. Only the JetSet best probe set of eEF1A2s was chosen to obtain Kaplan-Meier plots, where the number-at-risk is indicated below the main plot.

Protein immunohistochemical staining analysis

The data related to each protein are arranged in the database (The Human Protein Atlas:https://www.proteinatlas.org/humanproteome/pathology). The starting page is a summary interface, where the basic information of the protein is displayed. The different modules can be visited thereafter to collect the sets of data. The modules included in this database are: tissue expression profile, cellular localization profile, pathological expression profile and RNA expression profile.

GEPIA dataset

GEPIA (http://gepia2.cancer-pku.cn/) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from TCGA and the Genotype Tissue Expression (GTEx) projects, using a standard processing pipeline. GEPIA can profile the tissue-wise expression of one gene in different cancer types, using a dot plot.

Cell lines and plasmid

The human GC cell lines AGS (CL-0022) were kindly provided by Procell LifeScience & Technology Co., Ltd. (Wuhan, China), and the normal gastric cell line GES-1 were kindly gifted by the Ms. Juan An's research group (*Department of Basic Medicine Science*, *Qinghai University Medical College*). Mycoplasma testing has been carried out for the cell lines used; and the cell lines used have been authenticated. AGS and GES cells were maintained with 5% CO_2 at 37 ℃ in Dulbecco's modified Eagle Medium (DMEM) (Biosharp, Beijing, China). The knockdown sense sequence of GUF1, EFTUD2 and GSPT1 are provided in *Table 1*.

Table 1 The primer sequences

Gene	Direction	Primer sequences
GUF1	PF	ACATAAGCAACCAGTGGAGCCC
GUF1	PR	TCCAGCCAGCACCCAGAGC
EFTUD2	PF	CAGCATCGTTCAAGGTTTC
EFTUD2	PR	CATCAGACGAGGAGTAGCC
GSPT1	PF	CGTAGCCCCAGGTGAAAA
GSPT1	PR	AAATGTGCGTCCAGAATGA
GSPT2	PF	TTGGCTGTGCTGGTCATC
GSPT2	PR	GTTTTACCCCTGCCGTTT
GAPDH	PF	GAAGGTGAAGGTCGGAGT
GAPDH	PR	CATGGGTGGAATCATATTGGAA

Table 2 The primer sequences

si-RNA	Direction	Primer sequences
hGUF1	PF	CCUCCUAAAGUGCAUCGCAAATT
hGUF1	PR	UUUGCGAUGCACUUUAGGAGGTT
hEFTUD2	PF	GCCUCUCACAGAACCCAUUAUTT
hEFTUD2	PR	AUAAUGGGUUCUGUGAGAGGCTT
hGSPT1	PF	CCCGAUGAUGUAGAGACUGAUATT
hGSPT1	PR	UAUCAGUCUCUACAUCAUCGGTT

Cell transfection

Specific small interfering RNAs (siRNAs) targeting GUF1, EFTUD2 and GSPT1 (si-GUF1, si-EFTUD2 and si-GSPT1, respectively) and negative control siRNA (si-NC) were acquired from Beijing Xianghong Biotechnology Co., Ltd. (Beijing, China). For the siRNA experiments, 100 pmol siRNA and sample control (NC) were mixed with 125 µL serum-free medium and 4 µL Lipo8000 Transfection Reagent (Beyotime, Shanghai, China) and transfection was carried out. All transfected cells were cultured for 48 h before they were used for the various assays, unless indicated otherwise.

RNA extraction and quantitative real-time polymerase chain

Reaction

The total RNA from the cell lines was isolated using Total RNA Isolation Reagent (Biosharp), according to the manufacturer's instructions. The cDNA was reverse transcribed with 1 µg of total RNA, using the SPARKscript II RT Plus Kit (with gDNA Eraser, AG0304). The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the 2X SYBR Green qPCR Mix (with ROX, AH0104) with Roche LightCycler 96 instrument. Glyceraldehyde-3-phosphate dehydrogenase was used for normalization of the mRNA expression. All results were calculated using the $2^{-\Delta\Delta Cq}$ method. Each experiment was performed three times. The primer sequences are listed in *Table 2*.

Cell proliferation assay

Cell proliferation was examined through 5-ethynyl-2'-deoxyuridine assay. EdU staining was performed using BeyoClickTM EdU Cell Proliferation Kit with Alexa Fluor 555, in accordance with the manufacturer's instruction (C0075S, Beyotime).

Cell migration assays

To the chamber, 100 µL serum-free medium and 200 µL cell suspension (2.5×10^5/mL in serum-free medium) were added. Add 700 µL of culture medium (with serum) and it was incubated at 37 ℃ for 12–16 h. Subsequently, the medium was removed from the chamber and the chamber was washed twice in phosphate buffered saline (PBS). Following that, cells were fixed for 10 min with 4% paraformaldehyde at room temperature (15–20 ℃). Crystal Violet staining solution was used to stain cells for 10 min at room temperature, formaldehyde was removed, and the cells were washed with PBS twice. Finally, migrated cells were counted under a light microscope, and the number of migrated cells was calculated for each group.

Statistical analysis

Statistical analyses were performed using SPSS 26.0 (IBM, Armonk, NY, USA). A fold change >2 and P value <0.05 were used as the screening criteria to filter the differentially expressed genes. Two-tailed unpaired Student's *t*-test was used to compare the differences between the two groups. The Kaplan-Meier method and log-rank test were used for the survival rate analysis. For all analyses, a P value <0.05 was considered statistically significant. All measurement data were expressed as the mean ± standard deviation (SD) obtained from three independent experiments.

Results

Expression levels of GUF1, EFTUD2, GSPT1 and GSPT2 in patients with cancers

We compared the expression levels of *GUF1*, *EFTUD2*, *GSPT1* and *GSPT2* in cancer samples against those in normal samples using data from Oncomine databases. The analysis revealed that the expression of GSPT1 was mainly higher in bladder cancer, breast cancer, colorectal cancer (CRC) and myeloma samples as compared to that in the corresponding normal tissues, whereas the expression of *GSPT2* was higher in CRC and leukemia samples, but lower or not detected in other cancer samples. *GUF1* and *EFTUD2* were expressed higher in samples of CRC, lung cancer, GC, leukemia and lymphoma (*Figure 1A-1D*).

Relationship between the GUF1, EFTUD2, GSPT1 and GSPT2 mRNA levels and the clinical parameters of patients with GC

We used the GEPIA and TCGA datasets for the analysis and using $\log_2$ (TPM + 1) we derived the log-scale. Using the matched normal data, TCGA normal and GTEx data were matched, we compared the mRNA expression of GUF1, EFTUD2, GSPT1 and GSPT2 between GC and gastric tissues (*Figure 2*). The analysis showed higher expression levels of GUF1, EFTUD2 and GSPT1 in GC tissues than in normal tissues (*Figure 2A*). Furthermore, data from immunohistochemistry analysis (The Human Protein Atlas dataset) showed that the expression of GUF1, EFTUD2 and GSPT1 proteins was higher in the GC tissues than in the normal tissues (*Figure 2C,2D*). Furthermore, we compared the mRNA expression of GUF1, EFTUD2, GSPT1 and GSPT2 factors between GC and gastric normal tissues. The results indicated that the expression levels of GUF1, EFTUD2 and GSPT1 were higher in GC tissues than in normal tissues, and the expression level of GSPT2 had no statistically significant difference between GC and normal tissues. At the same time, GC tissues with high expression of GUF1, EFTUD2 and GSPT1 were disordered and lost its normal morphology (*Figure 2B*). Therefore, based on GC data in TCGA, we analyzed the expression level of GUF1, EFTUD2 and GSPT1 in AGS cell lines and GES control cell. The results revealed that mRNA levels of GUF1, EFTUD2 and GSPT1 were significantly higher in AGS, (P<0.001, *Figure 2D*). The mRNA levels of GUF1, EFTUD2 and GSPT1 were determined to be highly expressed in the AGS cell line.

Relationship between expressions of GUF1, EFTUD2 and GSPT1 with clinical profile

The correlation between *GUF1*, *EFTUD2* and *GSPT1* expression and clinical baseline data was analyzed. As the result of the analysis, statistically significant (P<0.05) differences were found between the *GUF1* expression and the tumor T-stage and patient's age and between *GSPT1* expression and tumor T-stage (*Table 3*).

Association of GUF1, EFTUD2 and GSPT1 mRNA Levels with the prognosis of patients with GC

The Kaplan-Meier method was used to assess the difference between "high" and "low" risk groups based on the best separation of *GUF1*, *EFTUD2* and *GSPT1* expression, and the correlation between the *GUF1*, *EFTUD2* and *GSPT1* mRNA levels and the survival of patients with GC was analyzed, the publicly available Kaplan-Meier dataset was used. The Kaplan-Meier curve and log rank test analysis revealed that the increased *GUF1* mRNA level was significantly associated with patients' OS, FP and PPS (P<0.05) (*Figure 3*). The increased *EFTUD2* mRNA level was significantly associated with OS (P<0.05) (*Figure 3*); however, it did not show the same association with FP and PPS (P>0.05) of all the patients with GC. The increased *GSPT1* mRNA level showed significant association with OS and FP (P<0.05) (*Figure 3*). The GC patients with high mRNA levels of *GUF1*, *EFTUD2* and *GSPT1* were predicted to have high OS, FP and PPS.

Relationship between the mRNA expression level of GUF1, EFTUD2 and GSPT1 clinicopathological parameters in GC patients

We analyzed the relationship between the mRNA expression of *GUF1*, *EFTUD2*, *GSPT1* and clinicopathological parameters of GC patients (individual pathologic stage, clinical T stage and individual primary therapy outcome) through TCGA. The mRNA expression levels of *GUF1*, *EFTUD2* and *GSPT1* were correlated with individual cancer stage: patients with a more advanced cancer stage tended to express higher mRNA levels of *GUF1*, *EFTUD2* and *GSPT1* (*Figure 4A-4I*). Similarly, the expression levels of *GUF1*, *EFTUD2* and *GSPT1* were significantly related to primary therapy outcome. The mRNA levels of *GUF1*, *EFTUD2* and *GSPT1* tended to be related with individual pathologic stage, clinical T stage and individual primary therapy outcome.

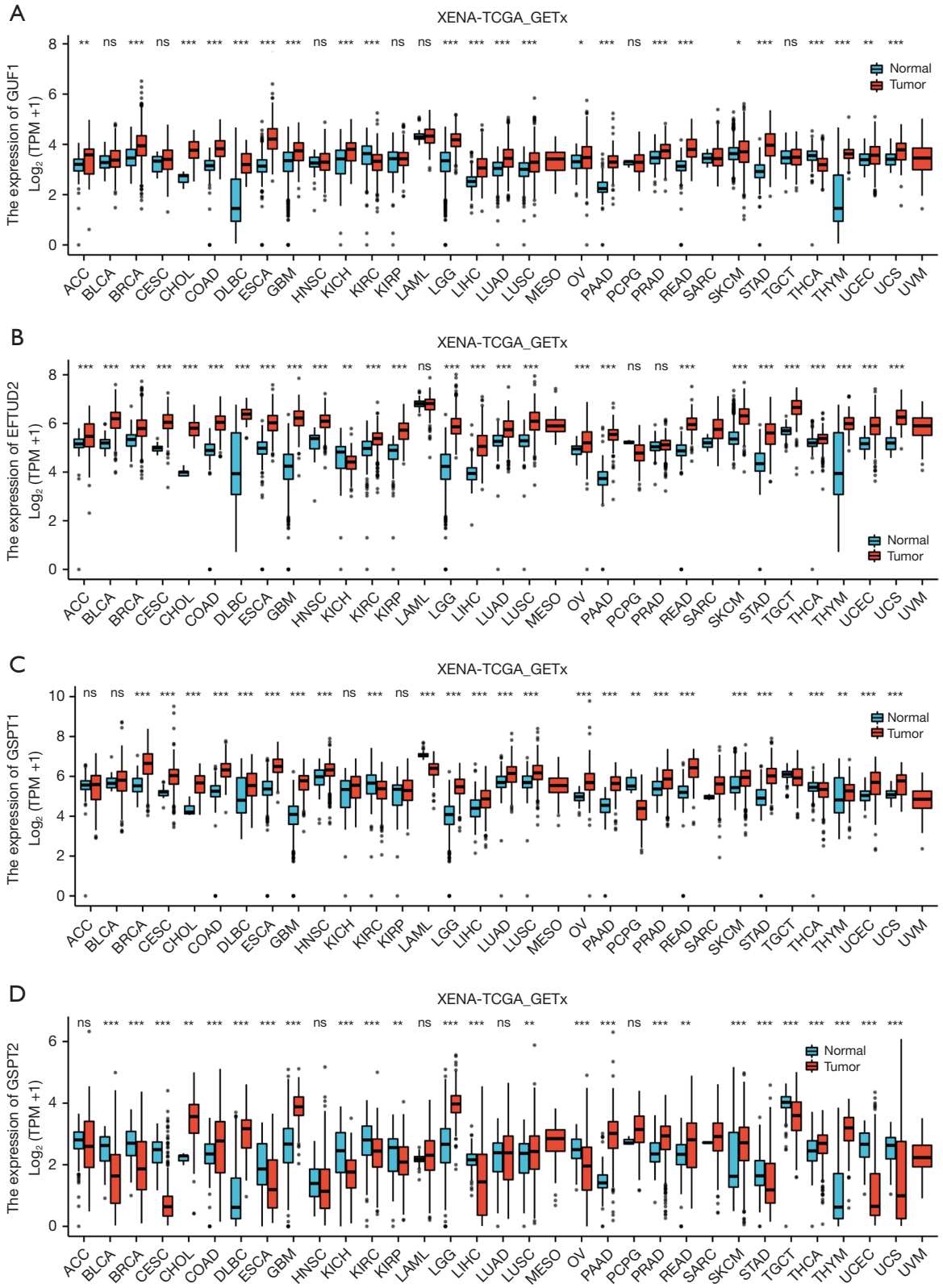
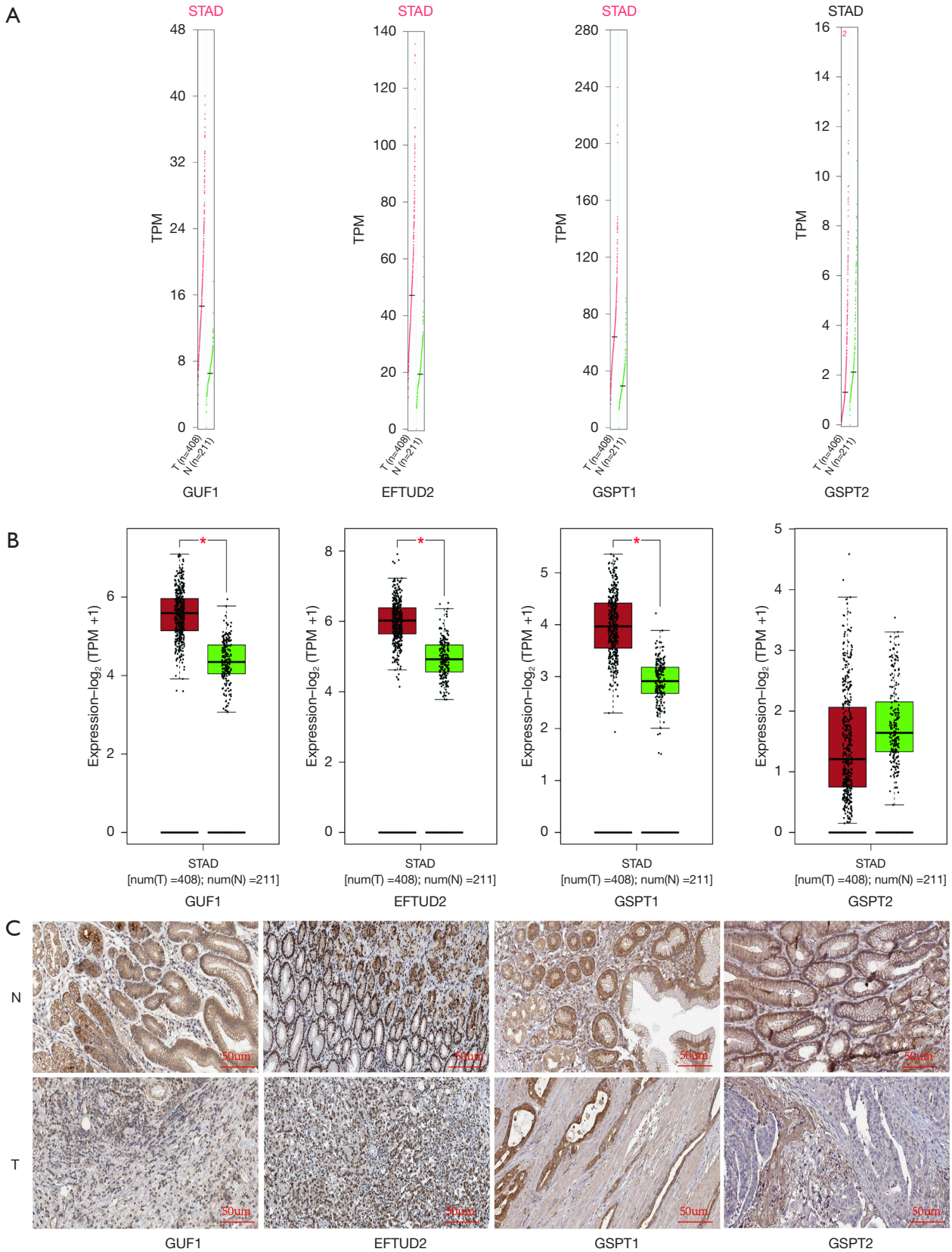


Figure 1 The expression levels of GUF1, EFTUD2, GSPT1 and GSPT2 in different types of cancers (from Oncomine and xiantao.love/products). ns, not statistically significant; *, P<0.05, **, P<0.01, ***, P<0.001 compared with normal samples. TPM, transcripts per million.



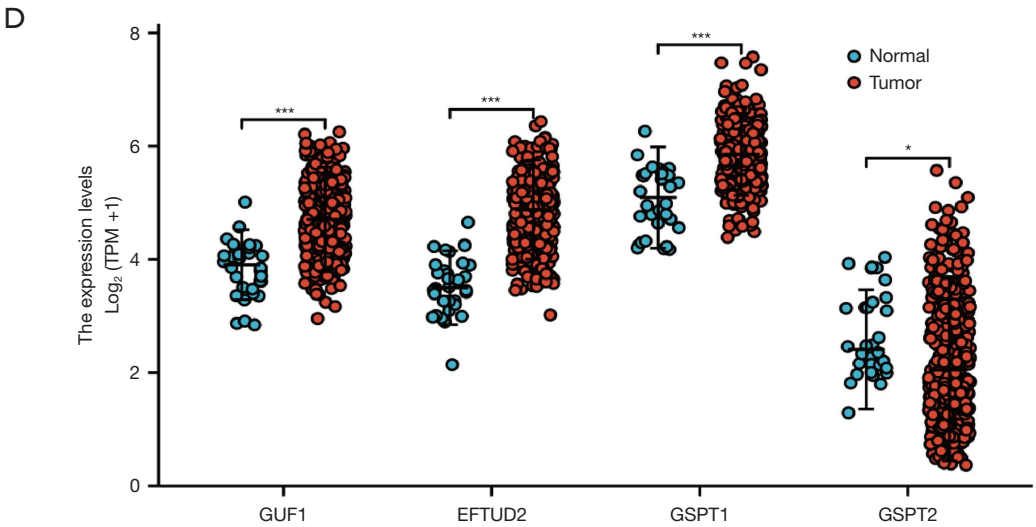


Figure 2 The expression of GUF1, EFTUD2, GSPT1 and GSPT2 in gastric cancer (GEPIA, TCGA). (A) Scatter diagram. (B) Box plot (red: cancer samples; green: normal samples). (C) Immunohistochemistry. The links to the individual normal and tumor tissues of each protein are provided for GUF1 (https://www.proteinatlas.org/ENSG00000151806-GUF1/tissue/stomach#img;https://www.proteinatlas.org/ENSG00000151806-GUF1/pathology/stomach+cancer#img), EFTUD2 (https://www.proteinatlas.org/ENSG00000108883-EFTUD2/tissue/stomach#img;https://www.proteinatlas.org/ENSG00000108883-EFTUD2/pathology/stomach+cancer#img), GSPT1 (https://www.proteinatlas.org/ENSG00000103342-GSPT1/tissue/stomach#img; https://www.proteinatlas.org/ENSG00000103342-GSPT1/pathology/stomach+cancer#img) and GSPT2 (https://www.proteinatlas.org/ENSG00000189369-GSPT2/tissue/stomach#img; https://www.proteinatlas.org/ENSG00000189369-GSPT2/pathology/stomach+cancer#img), respectively. Scale bar, 50 μm. (D) The expression level in gastric cancer (TCGA). *, P<0.05, ***, P<0.001 compared with normal tissue. TPM, transcripts per million; TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma.

Table 3 Relationship between GUF1, EFTUD2 and GSPT1 expression and GC clinical baseline profile characteristics

Parameter	Low expression	High expression	P
GUF1, n	187	188	
T stage	50.6%	49.3%	0.02*
N stage	49.8%	50.2%	0.87
M stage	48.7%	52.3%	0.59
Gender	49.9%	50.1%	0.72
Age, years, median [IQR]	64 [57, 71]	69 [61, 75]	<0.001*
EFTUD2, n	187	188	
T stage	50.1%	49.9%	0.10
N stage	49.6%	50.4%	>0.99
M stage	50.2%	49.8%	0.09
Gender	49.9%	50.1%	0.47
Age, years, median [IQR]	66 [57, 72]	68 [59, 74]	0.09
GSPT1, n	187	188	
T stage	50.1%	49.9%	0.004*
N stage	49.6%	50.4%	0.43
M stage	50.4%	49.6%	0.38
Gender	49.8%	50.2%	0.35
Age, years, median [IQR]	67 [58, 73]	68 [58, 73]	0.49

*, P<0.05 was considered statistically significant. GC, gastric cancer; IQR, interquartile range.

Diagnostic capability of GUF1, EFTUD2 and GSPT1 for GC

We analyzed the disease diagnostic capabilities of *GUF1*, *EFTUD2* and *GSPT1* expression in GC. Receiver operating characteristic (ROC) curves for each gene are shown in

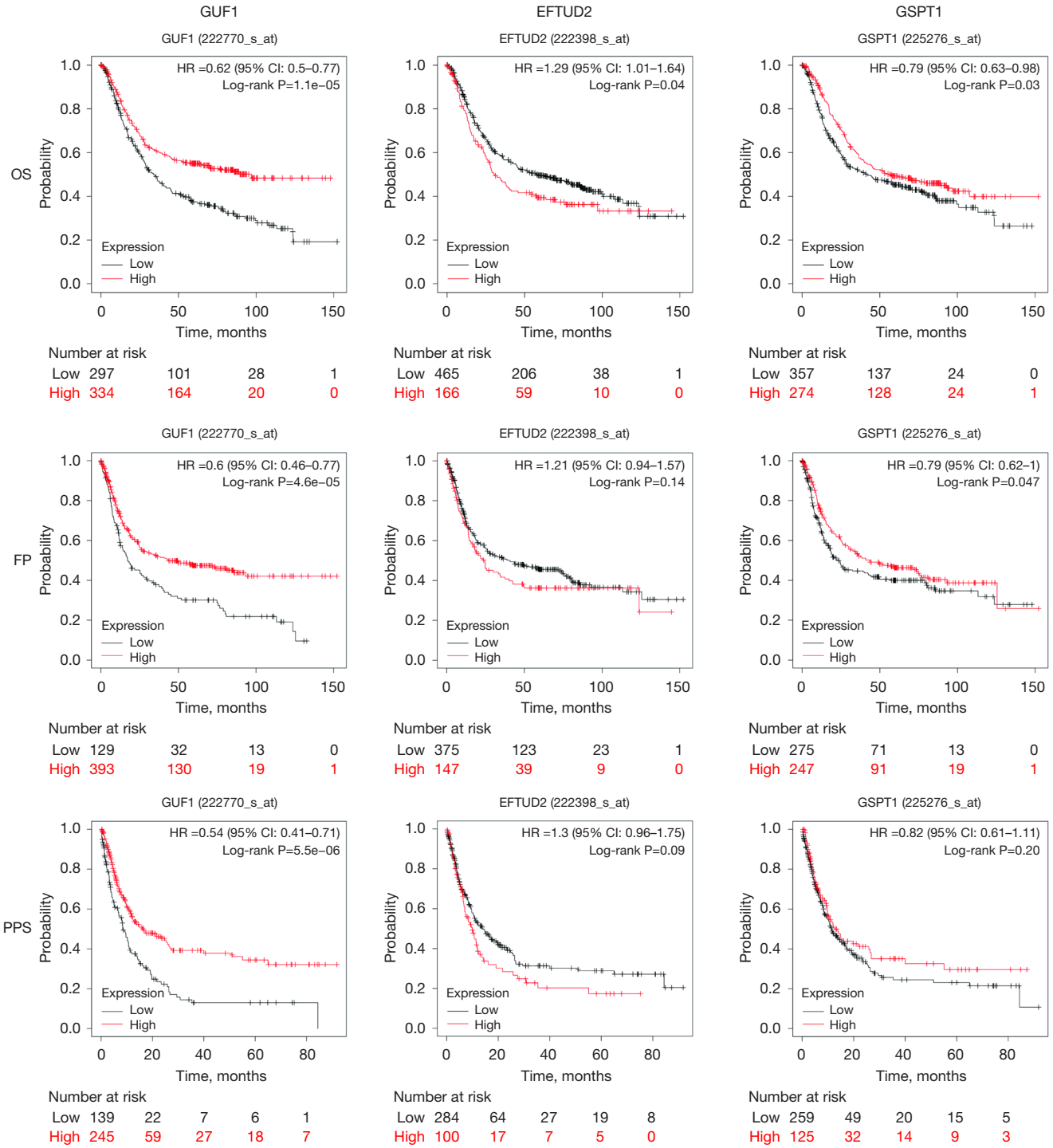


Figure 3 The prognostic value of expression level of *GUF1*, *EFTUD2* and *GSPT1* in gastric cancer patients (Kaplan-Meier plotter). OS, overall survival; FP, progression-free survival; PPS, post-progression survival; HR, hazard ratio; CI, confidence interval.

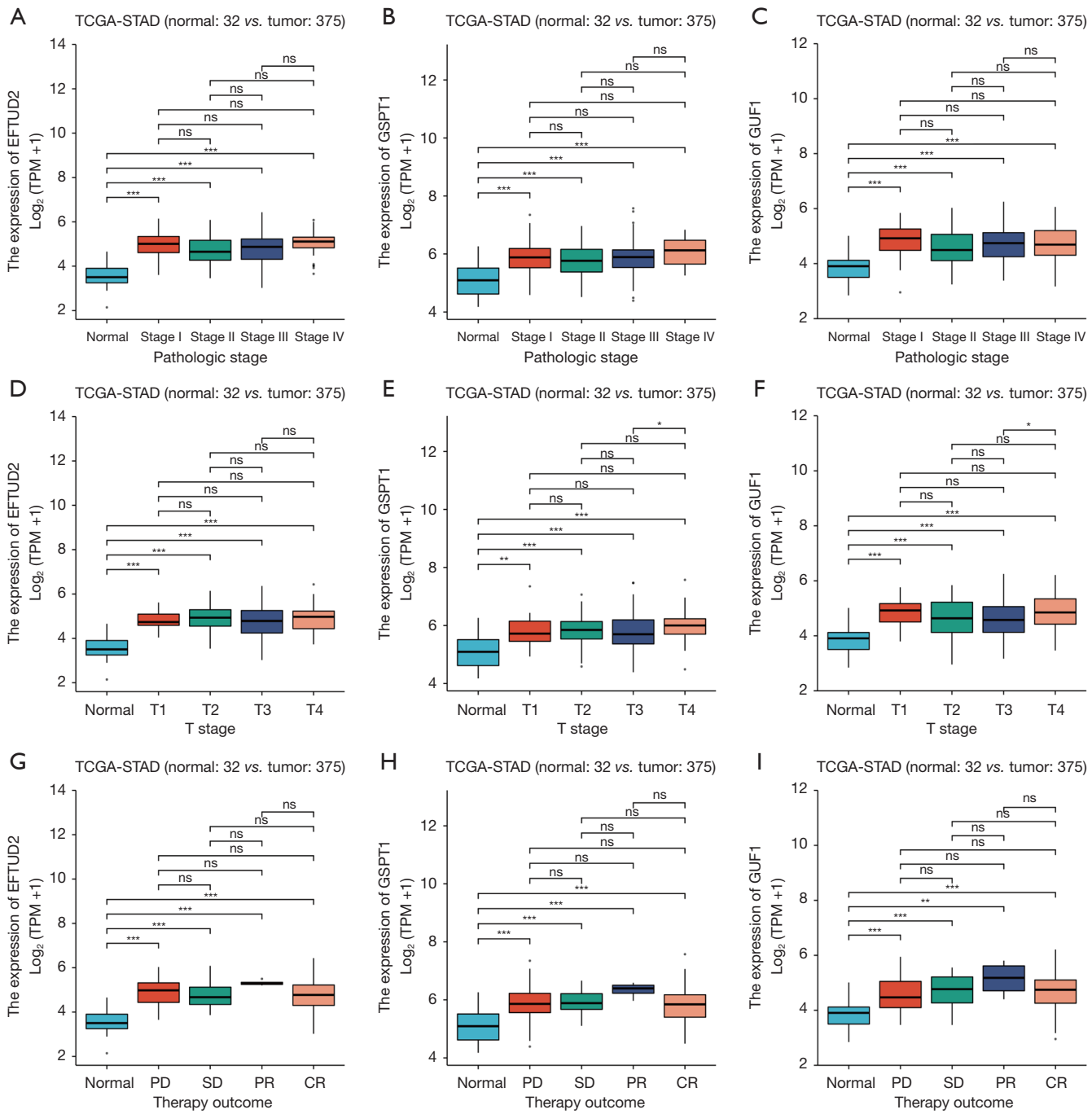


Figure 4 Relationship between the mRNA expression of *GUF1*, *EFTUD2* and *GSPT1* genes and individual cancer stage of gastric cancer patients. (A-C) The mRNA expression of the *EFTUD2*, *GSPT1* and *GUF1* genes were correlated with the patients' individual pathologic stage. (D-F) The mRNA expression of the *EFTUD2*, *GSPT1* and *GUF1* genes was correlated with the patients' clinical T stage. (G-I) The mRNA expression of the *EFTUD2*, *GSPT1* and *GUF1* genes were correlated with the patients' individual primary therapy outcome. ns, not statistically significant; *, P<0.05, **, P<0.01, ***, P<0.001 compared with normal tissue. TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma; TPM, transcripts per million; PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

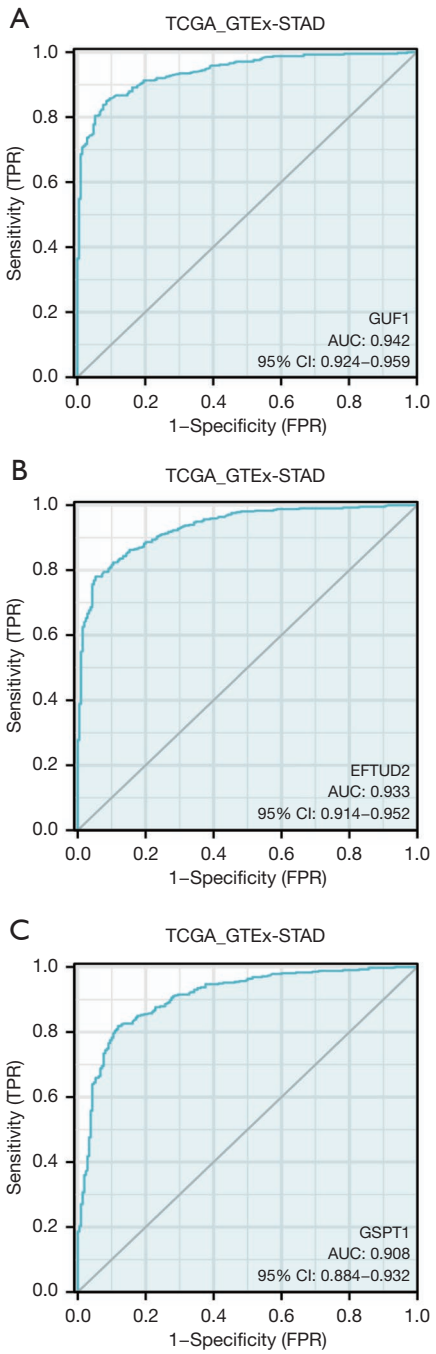


Figure 5 Diagnostic capability of GUF1, EFTUD2 and GSPT1 for GC. (A-C) Estimated ROC curves of the GUF1, EFTUD2, and GSPT1. All techniques can operate at levels where both sensitivity and specificity are >95%. Higher diagnostic accuracy is afforded by curves closer to the upper right corner. GC, gastric cancer; TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma; TPR, true positive rate; FPR, false positive rate; ROC, receiver operating characteristic; AUC, area under the ROC curve; CI, confidence interval.

Figure 5. Please note that the sensitivity range had been limited to show only values ≥90% and (1 – specificity) limited to show only values ≤20% to focus on high sensitivity and specificity operational conditions that would be suitable for the ROC triage application. The result showed that the expression of *GUF1*, *EFTUD2* and *GSPT1* genes could be called as early diagnostic markers for GC.

Predicted functions of GUF1, EFTUD2 and GSPT1 in GC cells

GUF1, *EFTUD2* and *GSPT1* expression level in cell lines and cell transfection

Figure 6A shows that *GUF1*, *EFTUD2* and *GSPT1* were highly expressed in AGS than in GES cell line, and the difference was statistically significant (P<0.05). For the RNAi experiments, according to the instructions of siRNA manufacturer, we tested the 100, 50, 30, 20, and 10 nM siRNA concentrations. As shown in *Figure 6A*, we performed siRNA-mediated silencing of *GUF1*, *EFTUD2* and *GSPT1*. Compared to control si-NC (nonsense siRNA), si-GUF1, si-EFTUD2 and si-GSPT1 (transfected with specific siRNA) reduced the mRNA levels of *GUF1*, *EFTUD2* and *GSPT1*, respectively over 60% gene silencing efficiency: indicative of efficient silence of the corresponding genes in AGS cells (*Figure 6B-6D*).

GUF1, *EFTUD2* and *GSPT1* knock-down suppressed cellular proliferation of cancer cell

We evaluated the changes of DNA synthesis in AGS cells with EdU immunofluorescence assay after GUF1, GSPT1 and EFTUD2 silencing, and cell proliferation was evaluated by CCK-8 assay. The results showed that the proportion of the cells in the S phase in the si-GUF1, si-EFTUD2, and si-GSPT1 groups was distinctly lower than that in the si-NC group (*Figure 7A-7C*). At the same time, cell proliferation was inhibited (*Figure 7D-7F*). These results revealed that GUF1, EFTUD2 and GSPT1 had proliferation-promoting properties in GC cells.

Silencing of *GUF1*, *EFTUD2* and *GSPT1* inhibited AGS cell migration and invasion ability *in vitro*

Metastasis in GC is characterized by increased cell motility, angiogenesis, and epithelial-mesenchymal transition. We investigated the roles of GUF1, EFTUD2, and GSPT1 in advanced GC. The tumor cell migration and invasion abilities were examined with Transwell assay. GUF1, EFTUD2 and GSPT1 knockdown by si-RNA dramatically

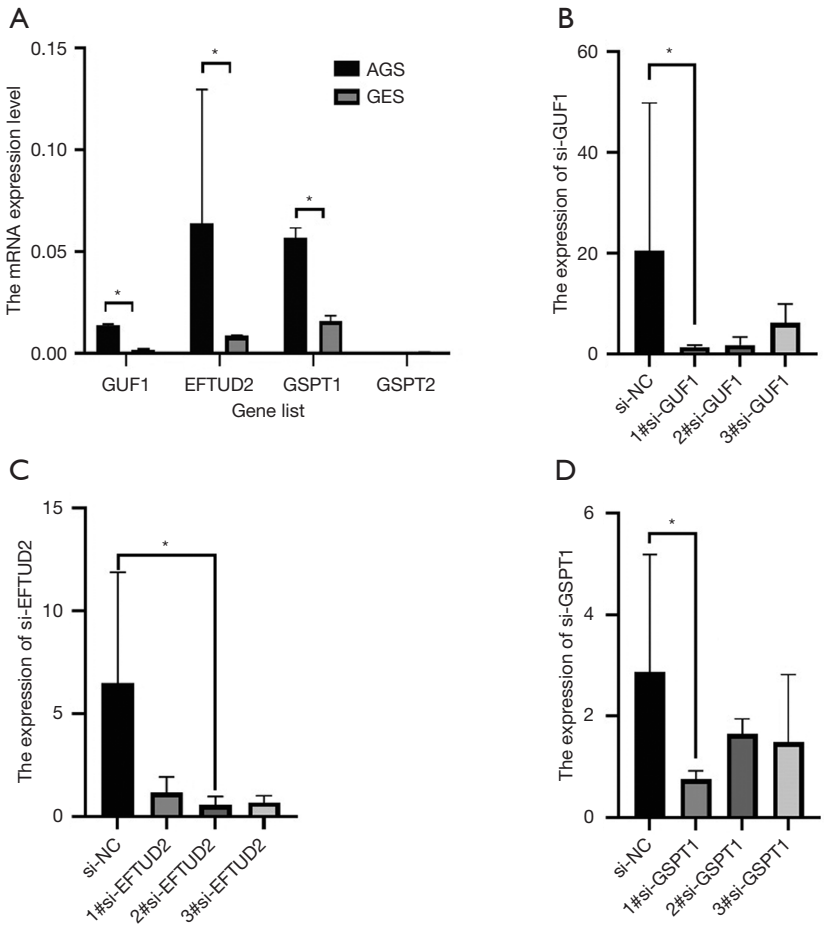


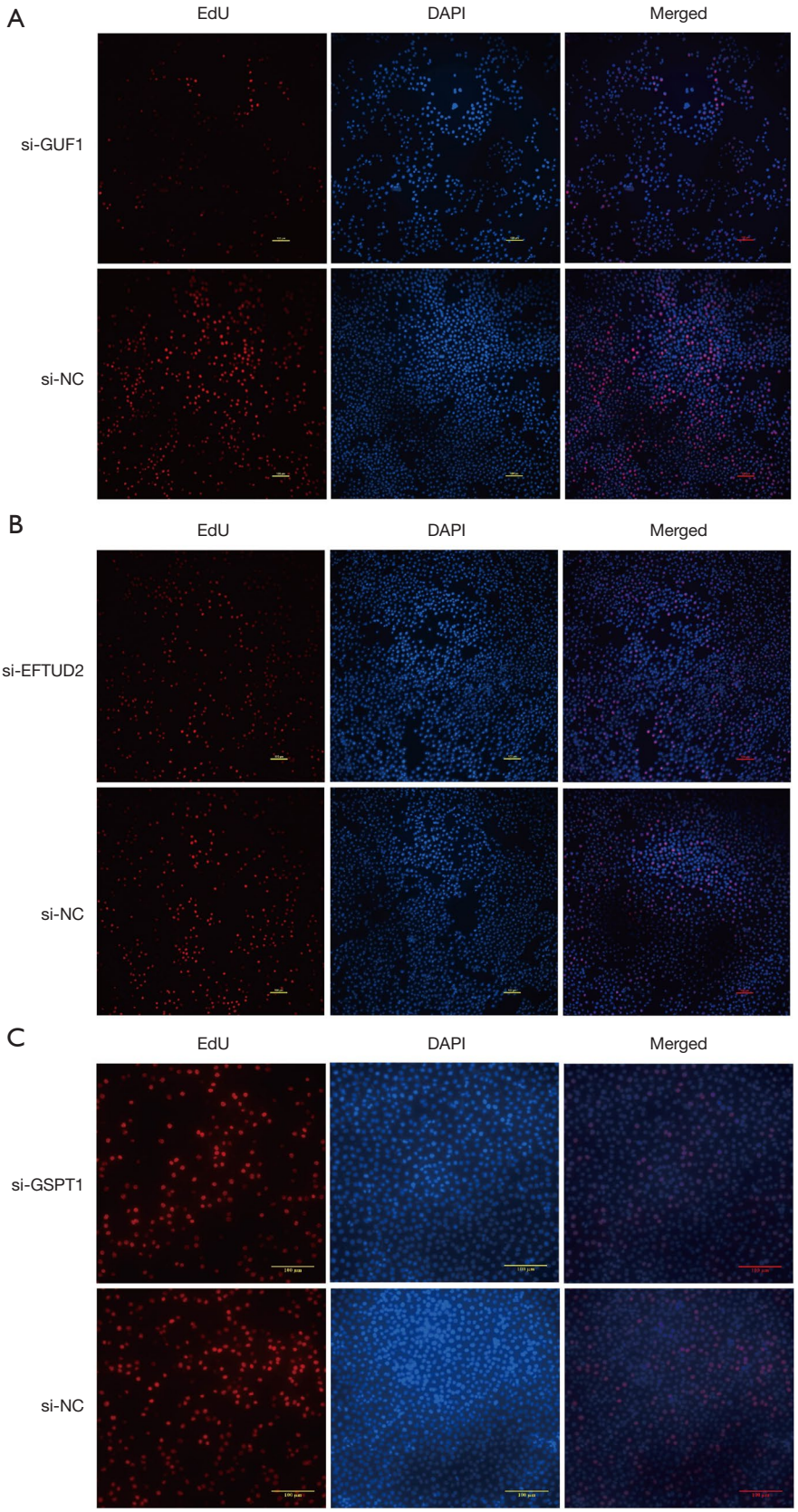
Figure 6 Transfection efficiency assay. (A) The expression level of *GUF1*, *EFTUD2*, *GSPT1* and *GSPT2* in gastric cancer cell lines. (B) GUF1 knockdown inefficiency detection. (C) EFTUD2 knockdown inefficiency detection. (D) GSPT1 knockdown inefficiency detection, *, P<0.05 compared with normal cell line.

reduced the invasion capacity of AGS cells compared to that of the si-NC group, suggesting that the knockdowns of GUF1, EFTUD2 and GSPT1 could inhibit the migration (*Figure 8A-8F*) and invasion (*Figure 8G-8L*) ability of GC cells.

Discussion

GC is one of the most fatal cancers worldwide (16,17), which manifests as highly prevalent malignant tumors in China. GC ranks fifth in cancer incidence and fourth in mortality worldwide (18). The 5-year survival rate for GC patients increased from 15.3% between 1995 and 1999 to 31.3% between 2005 and 2009, as screening became more common and treatment strategies improved (19). Despite the advancements in comprehensive treatment, including surgery, chemotherapy and radiation therapy, the prognosis of GC patients has not significantly improved. Currently, studies on the pathogenesis, occurrence and development of GC are facilitated by the modern molecular biology techniques.

The role of eEF1A2 in tumors remains debatable. Increased eEF1A2 expression may lead to an overall increase in protein translation. An increase in bulk protein synthesis may enhance cell replication because cell division requires sufficient protein production to fulfill the metabolic and size requirements of two new daughter cells (20). Increasing bulk protein abundance may decrease the time required to translate the overall mass of proteins necessary for cell division. If this is the case, then it would be expected that anything increasing protein translation rates would be predicted to be oncogenic. The reverse is certainly true, and



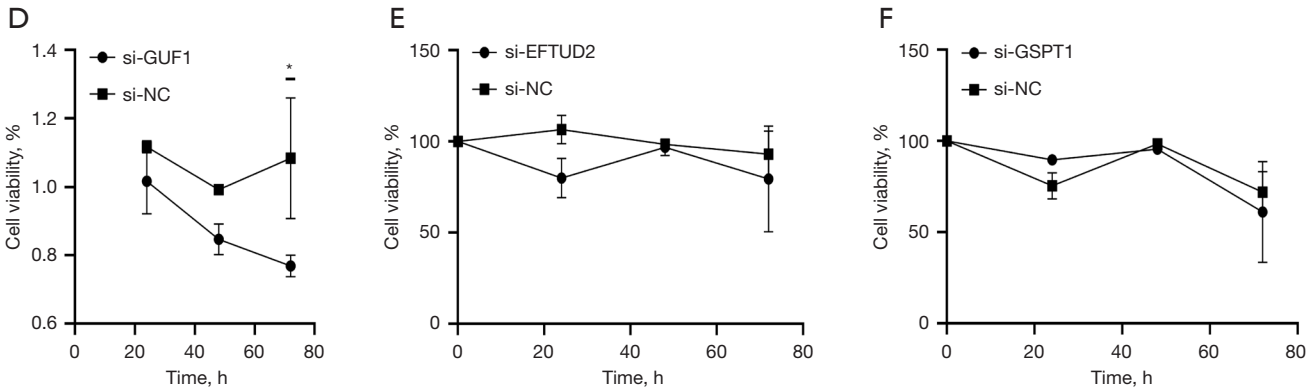
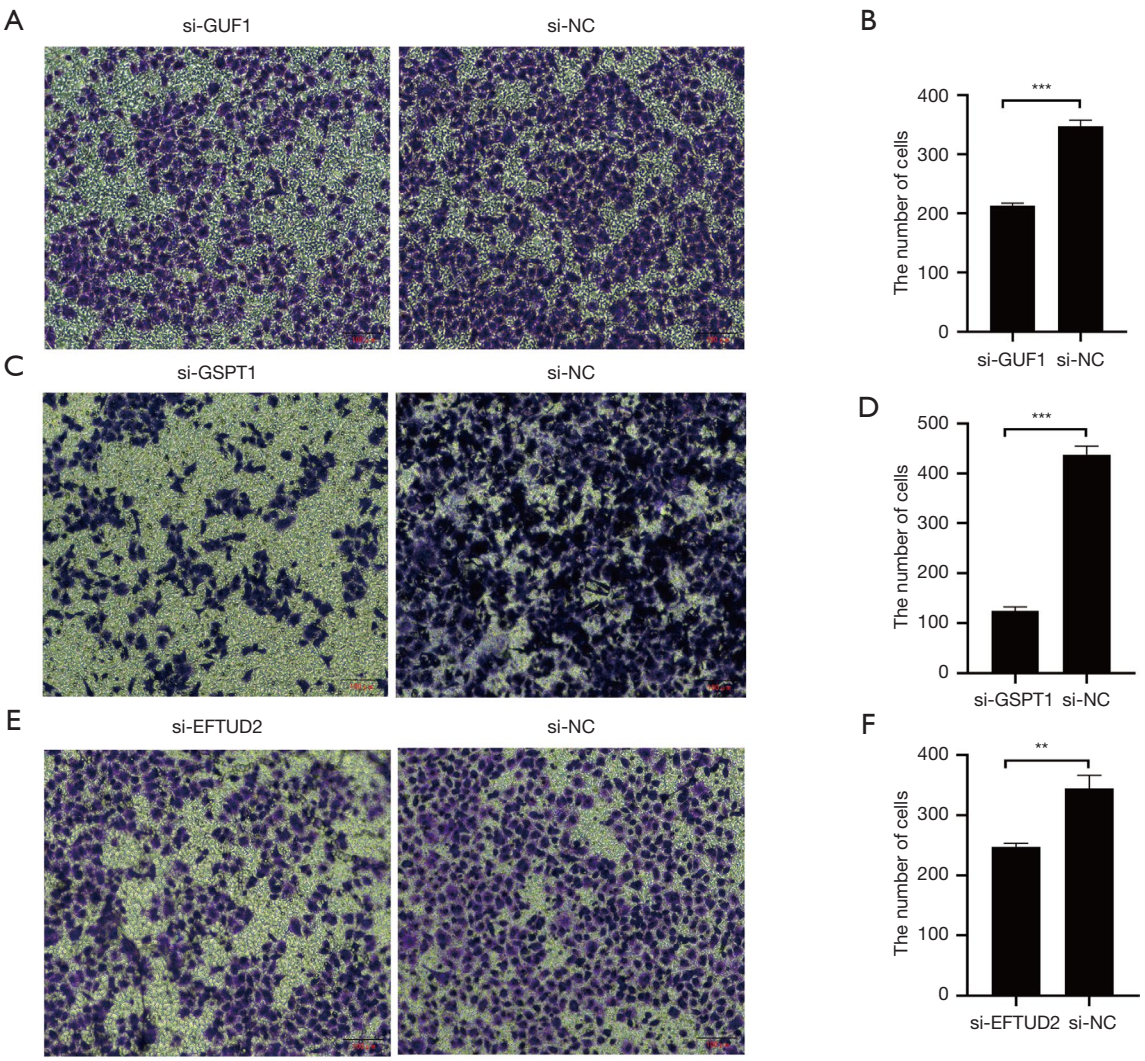


Figure 7 Effect of *GUF1*, *EFTUD2* and *GSPT1* knock-down on proliferation of gastric cancer cells. (A-C) Representative images of EdU staining (red) on day 2 to evaluate cell proliferation; nuclei were counter stained with DAPI (blue). Scale bar, 100 μm, ×10. (D-F) Representative images of CCK-8 experiments at 24, 48, and 72 h. *, P<0.05 compared with si-NC group.



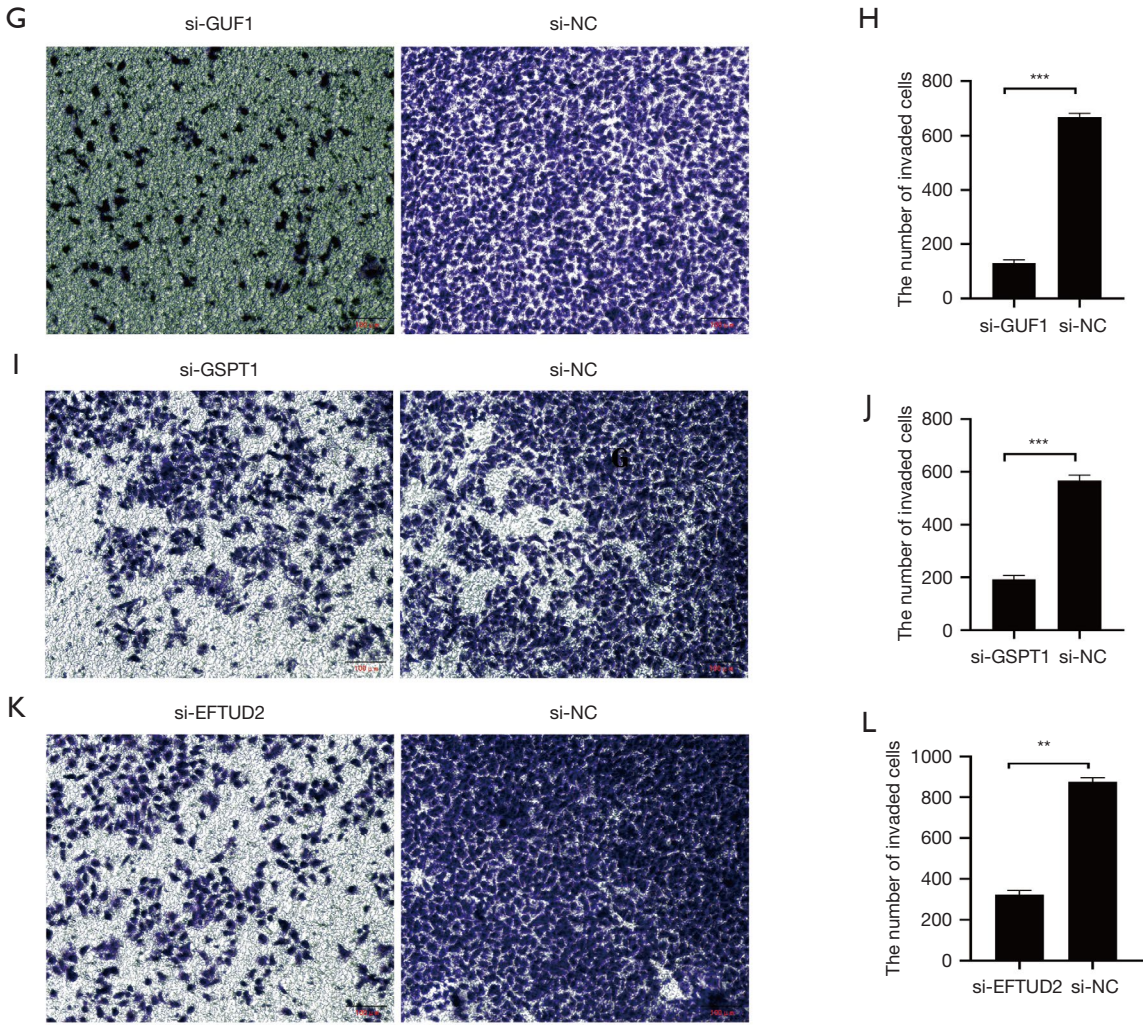


Figure 8 Silencing of *GUF1*, *EFTUD2* and *GSPT1* genes inhibit AGS cell migration and invasion ability *in vitro*. (A-F) Representative images of the cell migration ability detected by Transwell assay. (G-L) Representative images of the cell invasion ability detected by Transwell assay. Crystal violet staining, scale bar, 100 μm, ×40. **, P<0.01, ***, P<0.001 compared with si-NC group.

inhibitors of protein translation are universally and highly toxic to cells and organisms. The molecular mechanism underlying its role in tumorigenesis and progression is not clearly understood.

Although GUF1 was identified several years ago, its physiological role is still unclear. GUF1 had once been reported as prognostic markers for thyroid cancer (21). Furthermore, by using the least absolute shrinkage and selection operator (LASSO) regression model, Li and colleagues identified GUF1 for its contribution to a high probability of liver metastasis of CRC (22). We investigated the expression level of GUF1 in GC tissues through online bioinformatics tools and explored its relationship with the pathological features of GC. Analysis of data from Oncomine and The Cancer Genome Atlas datasets revealed that the expression of GUF1 in human GC samples was higher than in normal tissue. Similar result was obtained through our study in which GUF1 was expressed more in GC cells than in control cells. Moreover, GUF1 expression also correlated with clinical characteristics of the patients with GC. Our analysis using the Kaplan-Meier Plotter showed that a high GUF1 expression was significantly associated with OS, FP and PPS in all the patients with GC. In our Transwell assay, knockdown of the GUF1, EFTUD2 and GSPT1 could inhibit the migration ability of GC cells. Additionally, high GUF1 expression was significantly

associated with the age of patients. Therefore, GUF1 may be a new target for GC treatment.

EFTUD2 is a highly conserved spliceosomal GTPase that plays a crucial role in diverse biological functions, including spliceosome activation (23) and immune responses (24,25). Meanwhile, it has been reported that deletion of EFTUD2 inhibits the association of endogenous proteins, leading to increased apoptosis in breast cancer cells (26). Spliceosome is responsible for removing noncoding introns from mRNA precursors and generating mature mRNA, therefore, it plays an important role in almost all life processes (27). A study (28) identified that EFTUD2 plays a key role in regulating microglial polarization and homeostasis possibly through the NF-κB signaling pathway. Previously, there was little known about the molecular function of EFTUD2 in GC development. In this study, we identified the exact role and related mechanism of EFTUD2 in GC. We observed that EFTUD2 was upregulated in GC tissues compared to adjacent nontumor. In addition, we found that EFTUD2 was an independent prognostic factor for GC patients. A high level of EFTUD2 expression predicted a shorter overall and recurrence-free survival time in GC patients.

Interestingly, we found that EFTUD2 likely plays a role in maintaining the survival of GC cell lines. Our results indicated that a transient knockdown of EFTUD2 with siRNA inhibited cell viability. Our data indicated that EFTUD2 knockdown decreased the cell proliferation and migration *in vivo*. Further study to investigate the molecular mechanism of possible link between cell proliferation and migration regulated by EFTUD2 in GC is warranted. A study of EFTUD2 revealed that it plays a pivotal role in hepatocellular carcinoma (HCC) cell proliferation and the cell cycle (29), meanwhile another reported (30) that EFTUD2 as a novel oncogene helps to maintain the survival of HCC cells and promotes HCC progression. The high level of expression of EFTUD2 in HCC tissues indicates shorter overall and recurrence-free survival in HCC patients. These findings have similarities to our observation that EFTUD2 had proliferation-promoting properties in GC cells, which suggests that EFTUD2 maybe a new target for GC therapy in the future.

GSPT1 and GSPT2 are small GTPases which were initially found essential for the G1 to S phase transition of the cell cycle and later reported to function as a polypeptide chain release factor 3 (eRF3). The functions of eRF3 include the regulation of cell cycle phase-shifting from G1 to S phase (31), involvement in mRNA degradation, and ribosomal recycling (32,33), and it occurs in two isoforms, eRF3a and eRF3b, encoded by GSPT1 and GSPT2 genes, respectively. GSPT1/2 associates with eRF1 to mediate stop codon recognition and nascent protein release from the ribosome (34). Malta-Vacas *et al.* previously reported (35) that the expression level of eRF3a/GSPT1 was significantly high in intestinal gastric tumors. In addition, Tian (36) explored the potential effect of eRF3a/GSPT1 on gastric carcinogenesis and found that the expression level of eRF3a/GSPT1 was significantly increased in GC tissues, which is similar to the result of our observation. In addition, GSPT1 has been found to be overexpressed and oncogenic in a number of cancers (37), including gastric (35) and breast cancers (38). A study (39) has shown that GSPT1 plays a tumor-promoting role in the occurrence and development of colon cancer through specific signaling pathways. In the present study, high GSPT1 expression was significantly correlated with OS and FP in all patients with GC, establishing the oncogenic role of GSPT1 in GC.

Notably, it has been reported that GSPT1 promotes the proliferation, invasion, and migration of non-small cell lung cancer (NSCLC) cells, enhances tumorigenicity, and promotes the progression of lung cancer (40,41). In GC tissues, GSPT1 is highly expressed, miRNA-144 expression is down-regulated, and GSPT1 expression is significantly increased. By inhibiting miRNA-144, GSPT1 over-expression can promote the proliferation, invasion, and migration of GC cells, thereby promoting GC progression, which is consistent with the role of GSPT1 in tumors (42). It has also been reported that the overexpression of GSPT1 is related to the specific expression of GGCn alleles in various cancer cells, and that it is a potential oncogene (43). It could be argued that because all cells require protein synthesis, inhibitors of protein elongation would have substantial cytotoxic effects on normal tissues. However, rapidly growing tumor tissue may be more sensitive to decreases in protein synthesis than normal tissue because of the added burdens of an increased proliferation rate. Perhaps, when eEF1A2-inactivating agents are found, they might have efficacy in GC.

Tumor microenvironment (TME) refers to a complex and rich environment composed of multiple cells, which plays an important role in the growth and development of tumors. TME includes primary tumor cells, a variety of tissue cells (such as endothelial cells and fibroblasts), various immune cells, adipocytes, and extracellular fluid, which make TME a highly complex local environment. TME is an important component of tumor growth and development,

and has an important impact on the growth, metastasis, and drug resistance of tumor cells (44). In this study, GSPT1 GUF1, EFTUD2, and GSPT1 were highly expressed in GC tissues, and the knockdown of GUF1, EFTUD2, and GSPT1 could inhibit the proliferation, migration and invasion of GC cells. We hypothesize that the GSPT1 gene plays a promoting role in the growth of GC tumor cells. The goal of future drug development can be to inhibit the expression of GUF1, EFTUD2, GSPT1, which may overcome many of the current limitations in the diagnosis and prognosis of cancer patients, and can improve the specificity and effectiveness of current tumor treatments, and provide new strategies and ideas for personalized treatment and comprehensive treatment of cancer patients.

Conclusions

In summary, we determined that GUF1, EFTUD2 and GSPT1 have diverse functions and complex regulatory mechanisms in cancer. The mechanism in tumors has not been studied to a great degree yet. However, there is no doubt that GUF1, EFTUD2 and GSPT1 play an important role in GC tumorigenesis and development and may be potential targets for clinical diagnosis and treatment of GC.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-125/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-125/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-125/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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