Mathematical Biosciences and Engineering

MBE, 19(12): 12581–12600.
DOI: 10.3934/mbe.2022587
Received: 06 July 2022
Revised: 12 Aug 2022
Accepted: 18 Aug 2022
Published: 29 Aug 2022

http://www.aimspress.com/journal/MBE

Research article

Family with sequence similarity 153 member B as a potential prognostic biomarker of gastric cancer

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Abstract: Gastric cancer (GC) is one of the most common digestive tumors in Northwest China. Previous sequencing analysis revealed that family with sequence similarity 153 member B (FAM153B) might be the primary driver gene of GC. In this study, we aim to explore the potential roles of FAM153B in GC. Microarray data were firstly obtained from public databases with the aim to evaluate the genetic expression of FAM153B between GC and normal tissues. The results were verified in immunohistochemistry (IHC). We also performed the co-expression network analysis and enrichment analysis to identify underlying mechanisms. A correlation analysis of FAM153B expression and immune infiltration was performed then. Furthermore, two GC cell lines were used to evaluate the effect of FAM153B on gastric cell proliferation by employing MTT and Edu assays. Our findings suggest that FAM153B is downregulated in tumoral tissue, and positively associated with unfavorable survival. The enrichment pathways of FAM153B were regulation of signaling receptor activity, DNA replication, cell cycle transition, chromosomal regulation, and so on. Besides, from the perspective of bioinformatics, the protein expression level of FAM153B is related to the degree of immune cell infiltration. In vitro, overexpression of FAM153B inhibit the proliferation of two cell lines. In summary, this study demonstrates that FAM153B might serve as an effective prognostic and therapeutic biomarker in GC.

Keywords: FAM153B; gastric cancer; proliferation; immune; prognosis

1. Introduction

Gastric cancer (GC) is one of the most common digestive malignancies, and the incidence of GC gradually increased in recent years [1]. As the symptoms of early-stage GC may not be obvious, most patients with GC are detected in late and inoperable stages. Despite great progress in multimodal therapy, 5-year survival rates of patients with advanced GC are still less than 30% [2–4]. GC has emerged as a serious health problem and becomes a major burden worldwide, especially in East Asia [5,6]. Tumor biomarkers could serve as integral markers of pathogenic processes, disease activity, and therapeutic drug response. Advances in omic technologies have made more and more cancer-specific biomarkers been discovered, including DNA, RNA, exosome, etc. Rapid development in cancer biology has been tremendously promoted by the selection of appropriate biomarkers [7]. Distinct profiles in human cancers make it challenging in disease diagnosis, survival prediction, and treatment. Therefore, exploiting new molecular targets and prognostic biomarkers for GC become an urgent priority for disease management.

Previously, we performed genome sequencing and bioinformatic analysis of gastric cancer tissues, thereby identifying several potential driver genes (data were not shown). Family with sequence similarity 153 member B (FAM153B) is a member of FAM153, and this family includes several coding proteins with unknown functions. FAM153A and FAM153C are the other two members of the FAM153 gene family. The FAM153B gene is located on chromosome 5 and its full-length cDNA is 1966 bp, which could encode protein (NP_0012525441). Its expression has been demonstrated in several monkeys and humans. Ferdouse et al. implemented a comprehensive genome-wide association study and demonstrated that FAM153B could be linked to lung disease [8]. However, the potential function and mechanism of FAM153B in cancers, especially gastric cancer, have not been reported. Thus, it is essential for comprehensive gene expression and functional analysis in GC. This will help elucidate diagnostic biomarker, prognostic indicator, and potential therapeutic target. Besides, this study also provides a rationale for further studies of gastric carcinogenesis.

Combination of experimental and bioinformatics method contributed greatly to this subject. In this study, we firstly obtained the genetic data for GC from the public database, evaluated the differential expression and prognostic power of FAM153B through bioinformatics and experimental methods. To assess the potential function of FAM153B in GC, a series of bioinformation analysis, including immune analysis and pathway-enrichment analysis, were performed using online database and R software. Finally, two GC cell lines was utilized to investigate the potential biological function in vitro.

2. Materials and methods

2.1. Data source

Transcriptome data of FAM153B in multiple cancers and normal tissues were retrieved from the UCSC Xena browser (https://xenabrowser.net/datapages/). All the expression data were converted to TPM format, and log2 transformed. The log2 fold change was set to 1, while the P-

value was set to 0.05.

2.2. Evaluation the diagnostic performance

The receiving operating characteristics (ROC) curves were plotted to evaluate the diagnostic value. The area under the ROC curve were calculated by using the pROC package. AUC $\geqslant$ 0.70 was considered as better discrimination, and enabled us to make disease predictions.

2.3. Tissue microarray analysis based on immunohistochemical (IHC) staining

The procedure involving human data in this study was approved by the Ethics Committee of the First Hospital of Lanzhou University (LDYYLL2021-251). Tissue microarray analysis (TMA) was purchased from Shanghai Outdo Biotech, containing 65 GC and 65 adjacent stomach tissue. All patients were aged between 40 and 78 years. None of the patients received any treatment prior to surgery. All patients had at least 5 years of follow-up data (Table A1). Individuals with missing clinical events were excluded. All formalin-fixed paraffin-embedded (FFPE) tissues were cut into sections with the core size of 5 μm. Subsequently, these sections were stained with primary antibody against FAM153B (Solarbio, China). Primary antibody was used at a dilution of 1:150. FAM153B staining score was classified according to staining intensity and percentage of tumor cells. The staining intensity was classified as 0–3+ together with expression degree (no, weak, moderate, and strong). Percentage of positive tumor cells was characterized as 1 + ($\leqslant$ 25%), 2 + (26–50%), 3 + (51–75%), and 4 + (> 75%). The final score is defined by the product of the score from 0 to 12, and the median score of 6 was set as the threshold to distinguish between high- and low- expression. Finally, we analyzed the relationship between FAM153B expression and overall survival of these patients.

2.4. Analysis of FAM153B and its co-expression genes in gastric cancer

The transcriptome data was firstly obtained from TCGA-STAD datasets. Subsequently, the modular analysis in LinkedOmics was performed to identify the co-expression genes of FAM153B in GC with FDR of 0.05. GSEA analysis was utilized to investigate the function of FAM153B. Besides, the top 50 positively/negatively correlated candidate genes were respectively used to construct the PPI network through GeneMINIA online analytical platform. Finally, the fundamental functions of these genes were explored with built-in functions of GeneMINIA platform.

2.5. Assessment of the tumor microenvironment

ssGSEA and GSVA were utilized to quantify twenty-four immune cell types. Subsequently, spearman correlation analysis was performed to explore the relationship between FAM153B expression and the composition of immune infiltrates.

2.6. Cell line and culture

Human GC cell lines (MKN45and HGC27) were purchased from the American Type Culture Collection, and were cultured in DMEM and RP1640 media respectively, supplemented with 10%

fetal bovine serum (FBS) and 1% antibiotics (streptomycin /penicillin). Cells were incubated at 37°C in a 5% CO_2 humidified environment.

2.7. Lentivirus transfection of MKN45 and HGC27 cells

The cDNA fragment of human FAM153B (NM_001265615.2) was firstly synthesized and cloned into a lentiviral expression vector carrying a flag tag accordingly by Shanghai GeneChem Co., Ltd. The empty vector was used as a negative control. The cells were plated in a 6-well plate at 50–70 % confluency. Then, MKN45 and HGC27 cells were transduced with lentiviral particles carrying plasmids containing FAM153B or empty vector, respectively. The stably transfected cells were selected by using puromycin. The efficiency of FAM153B overexpression was confirmed by Western blot analysis.

2.8. Cell proliferation assay

MTT and EdU were performed to evaluate the cell proliferation. The MTT Cell Proliferation and Cytotoxicity Assay Kit was purchased from Solarbio company. The cells were seeded into 96-well plates at a density of 5×10^3 cells/well. MTT dye (10 μl per well) was added into each well at different time points (0, 24, 48, and 72 h). The mixture was incubated for 4 h at 37 °C. Next, the absorbance of wells at 490 nm was measured with a microplate reader (Thermo Scientific, United States). The EdU assay was performed by Meilun EdU Cell Proliferation Kit with Alexa Fluor 555 (Meilun, China) according to the manufacturer's instructions. The EdU cell line was photographed by using the Olympus IX51 microscope (Olympus, Japan).

2.9. Western blotting

The total protein of GC cell lines was extracted by using RIPA buffer supplemented with phosphatase and protease inhibitors (Beyotime, China). Then, total proteins were collected and quantification was performed by using BCA kit (Solarbio, China). Total proteins were subjected to gel electrophoresis and sequentially transferred into a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). After overnight incubation with primary antibodies (Invitrogen, USA), they were followed by incubating with a secondary antibody (Immunoway, USA) for 1 h. Primary antibody was used at a dilution of 1:1000. Membranes were developed with ECL substrate (Beyotime, China) and exposed using film.

2.10. Statistical analysis

Graph Prism and R software were used for the statistical analysis. The differences between levels of FAM153B expression and clinical parameters were assessed by the chi-square test or Fisher-test. P-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Downregulation of FAM153B in GC tissues from public datasets and retrospective validation sets

Based on TCGA and GTEx databases, we evaluated the level of FAM153B expression in pan-cancer tissues. FAM153B was found to be downregulated in 25 tumor tissues, but upregulated in CHOL, LAML, and THYM (Figure 1A). As shown in Figure 1B, FAM153B was downregulated approximately 1-fold in GC. To further evaluate the diagnostic value of FAM153B in GC, we performed the ROC analysis and calculated the AUC value. As shown in Figure 1C, FAM153B showed the promising diagnostic efficacy. To validate the expression levels of FAM153B in GC and normal tissues, we examined changes in protein levels of FAM153B from clinical samples using IHC. The results showed that the expression of FAM153B was lower in GC tissue than that of the normal tissue (Figure 2A,B).

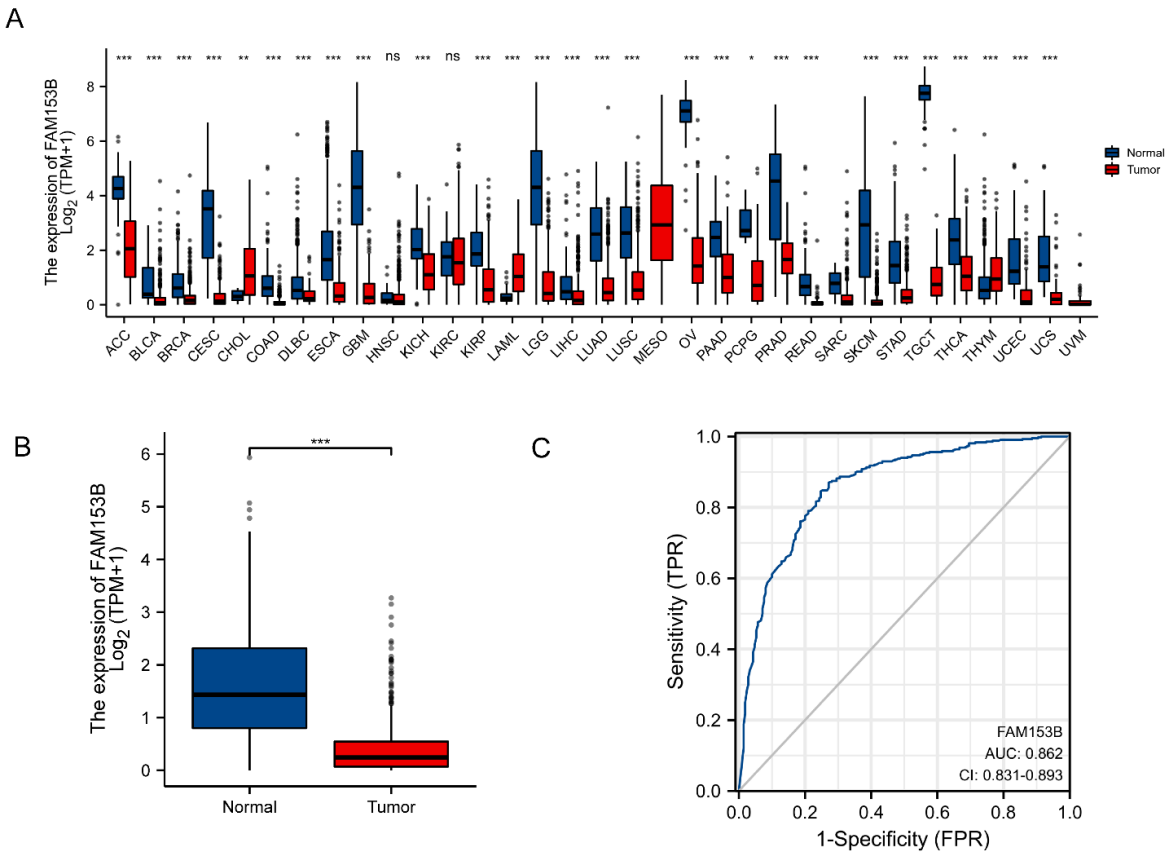

Figure 1. Downregulation of FAM153B in GC tissues using public database. (A) The mRNA expression level of FAM153B in the TCGA-GTEx database was analyzed. (B) The gene expression level of FAM153B in the TCGA cohort dataset was analyzed. (C) Diagnostic value of FAM153B in GC. (* P < 0.05, **P < 0.01, ***P < 0.001)

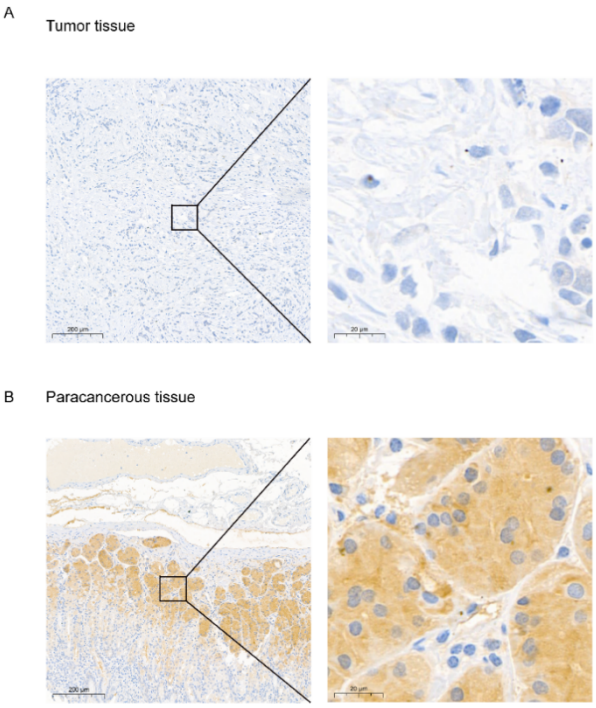

Figure 2. Protein expression of FAM153B in GC tissues (Figure 2A) and adjacent non-tumor tissues (Figure 2B) were detected by IHC (n = 65).

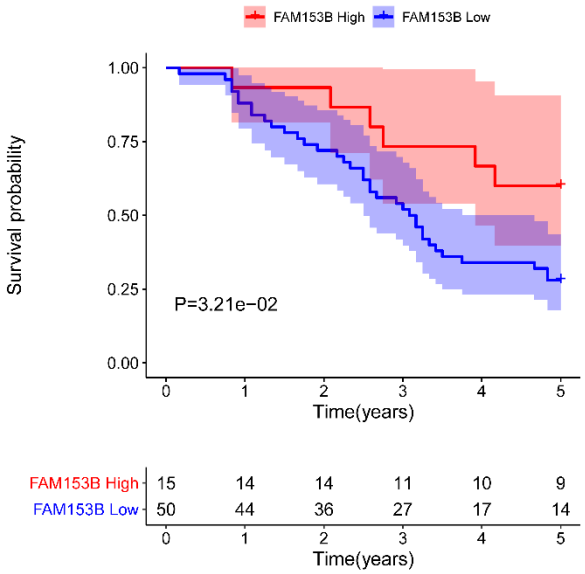

Figure 3. Kaplan-Meier analysis showed that the overall survival of GC patients with high or low FAM153B protein level.

3.2. Low FAM153B protein indicates poor overall survival of GC patients

Kaplan-Meier curve was plotted to evaluate the association between overall survival and FAM153B expression. GC patients having lower expression of FAM153B showed a significantly reduced OS (P = 0.032, Figure 3). Based on their IHC scores of FAM153B in cancerous tissues, these patients were grouped into high (n = 15) and low (n = 50) expression. Statistical analysis revealed that the protein levels of FAM153B showed close association with GC patients' clinical parameter of overall survival (Table 1).

Table 1. Relationship between FAM153B expression and clinical parameters of GC patients.

Characteristics		Low	High	P
	N = 65	N = 50	N = 15	
Age(years)	⩽65	37	12	0.636
	＞65	13	3	
Gender	MALE	36	11	0.919
	FEMALE	14	4	
Stage	I-II	25	8	0.821
	III-IV	25	7	
T	T1-2	13	7	0.128
	T3-4	37	8	
N	N0	14	3	0.536
	N1-3	36	12	
M	M0	44	13	0.890
	M1	6	2	
Differentiation	well-moderately differentiated	20	8	0.506
	poorly differentiated	26	7	
	unknown	4	0	
Tumor size	≥ 5 cm	14	4	0.758
	< 5 cm	33	14	
Tumor location	Proximal + middle	15	5	0.746
	Distal	32	13	
Treatment	Proximal + total gastrectomy	16	3	0.229
	distal gastrectomy	31	15	
Helicobacter pylori	Negative	16	7	0.262
	Positive	24	5	
	Unknown	10	3	
HER2 status	Negative	42	15	0.321
	Positive	6	0	
	Unknown	2	0	
Survival	Alive	14	9	0.023
	Dead	36	6	

3.3. Enrichment analysis based on co-expression genes of FAM153B in GC patients

In order to elucidate the biological significance of FAM153B, the change in co-expression of genes was obtained, and further involved in pathway enrichment analysis. We identified a total of 7084 positively correlated genes and 638 negatively correlated genes with statistically significant difference (FDR < 0.05). As shown in Figure 4, we presented a heatmap of the top 50 most relevant positive and negative genes, respectively. GSEA analysis indicated that these genes were involved in DNA replication-related pathway. The PPI network constructed around positive/negative genes reveals similarity between these genes. The PPI network of positive genes showed an enrichment in signaling activity-related genes, while the PPI network of negative genes showed an enrichment in DNA replication-related genes.

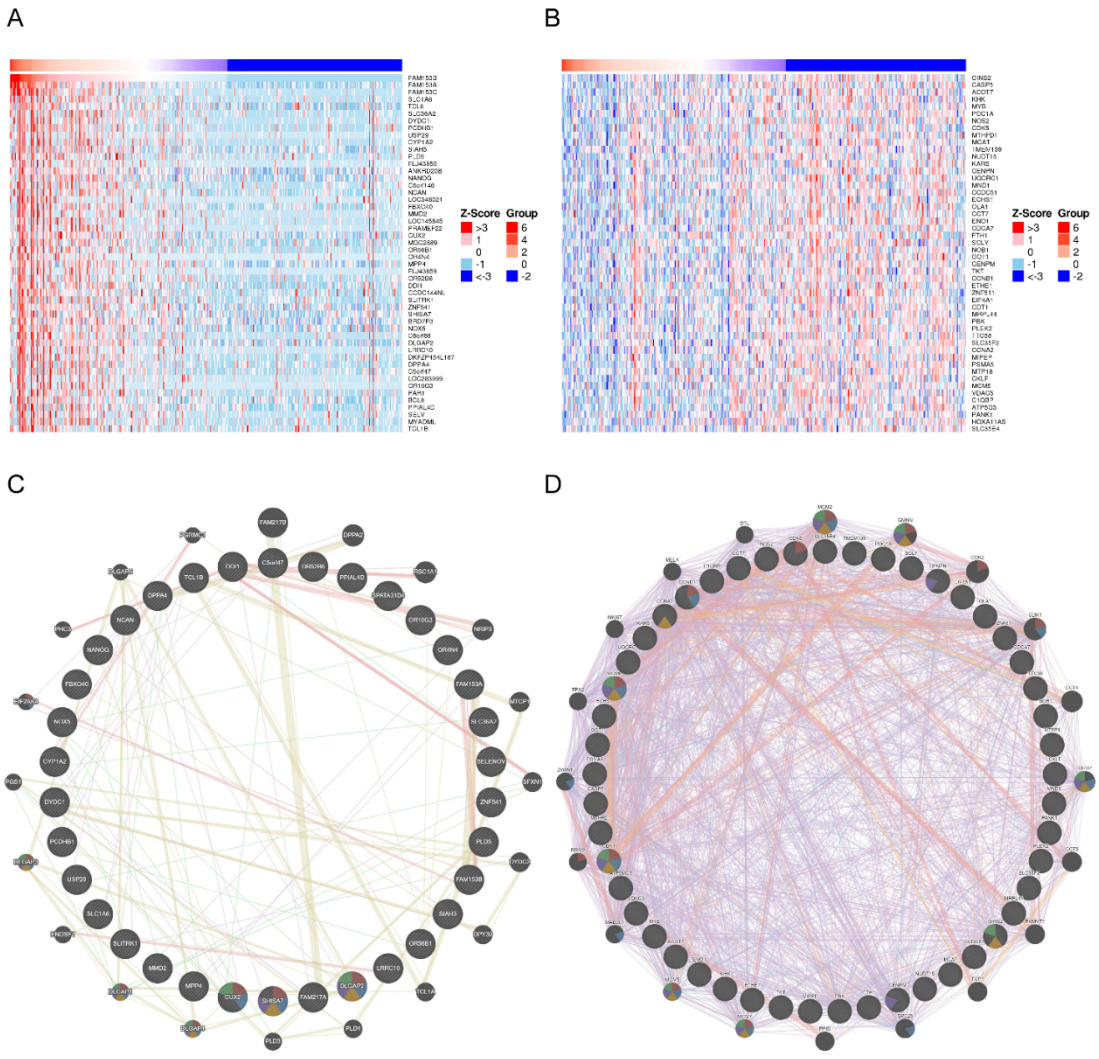

Figure 4. FAM153B co-expression genes and its relevant regulatory pathways in GC. (A, B) Heat maps show the top 50 positive and top 50 negative correlated genes of FAM153B in GC. (C, D) The biological functions of positive and negative correlated genes were evaluated using PPI network based on GeneMINIA.

3.4. Relationship between FAM153B expression and immune infiltration

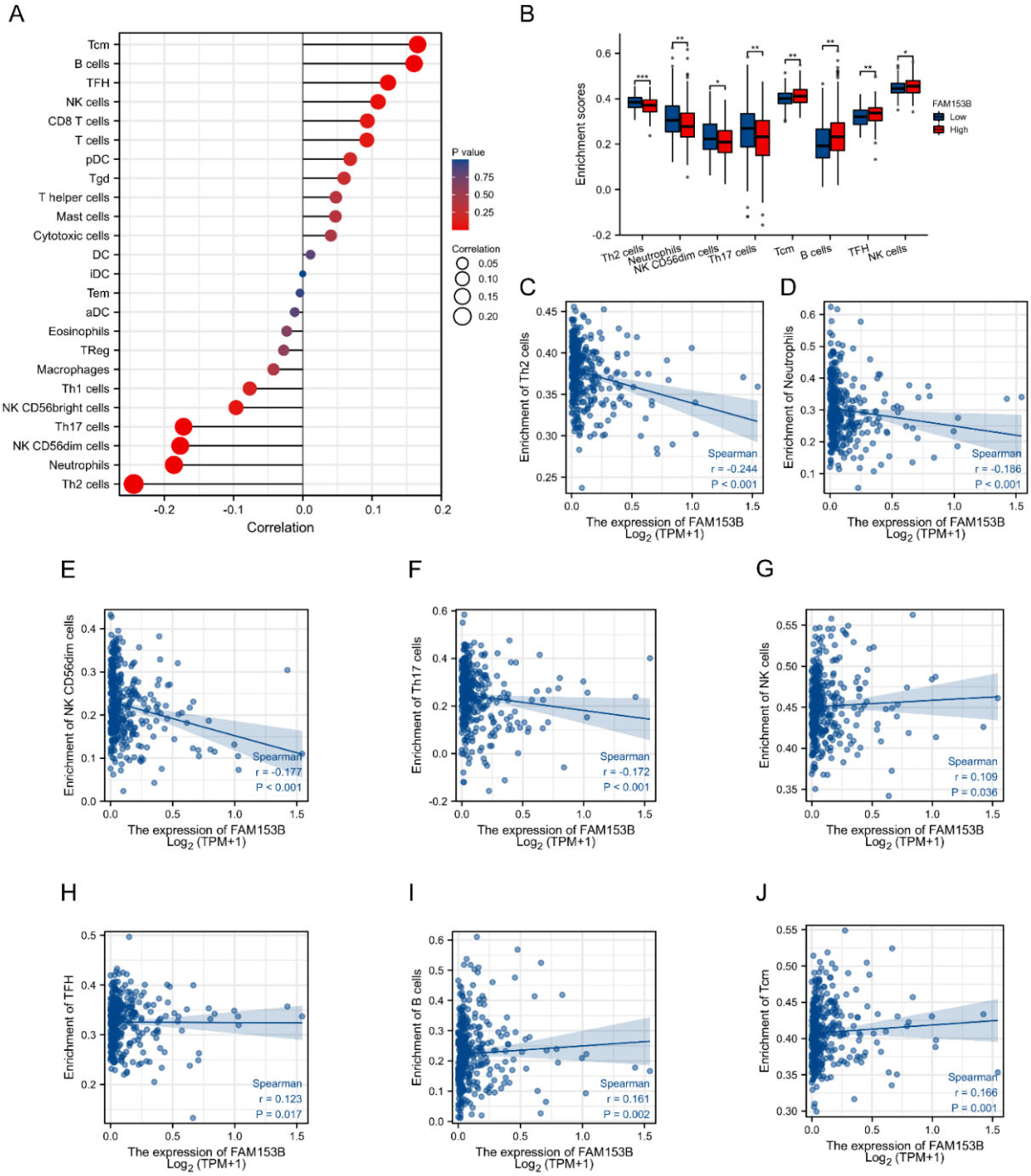

Figure 5. The expression level of FAM153B was correlated with immune cells infiltration in GC. (A) Correlation between the proportions of different immune cells and FAM153B expression levels. (B) The histograms of immune cells infiltration level related to the expression level of FAM153B. (C–J) Spearson's analysis showing the difference of immune cells infiltration level based on expression of the candidate protein.

The correlation between FAM153B expression and immune cell infiltration levels was determined by Spearman correlation analysis. As shown in Figure 5, the expression of FAM153B was positively correlated with immune cells (NK cells, TFH, B cells, Tcm) ($P < 0.05$), and negatively correlated with (Neutrophils, NK CD56dim cells, Th17 cells, Th2 cells) ($P < 0.05$). Analysis of FAM153B expression and corresponding immune cells indicated that FAM153B was significantly negatively associated with Th2 expression ($P < 0.05$).

3.5. Overexpression of FAM153B inhibited the proliferation of GC cells in vitro

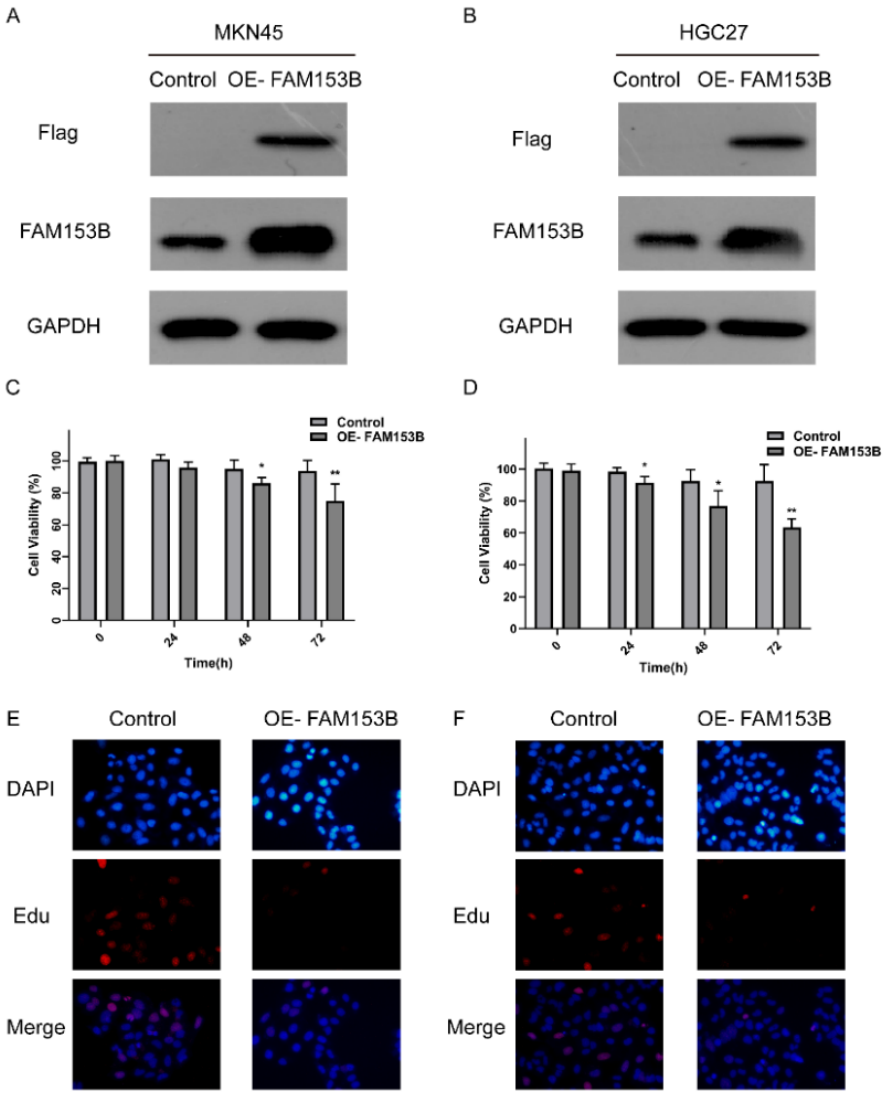

Figure 6. FAM153B inhibits gastric cancer growth in vitro. (A, B) Western blotting showing successful overexpression of FAM153B in gastric cancer cell lines (MKN45, HGC27). (C, D) MTT assays were performed to evaluate cell viability, indicating that two cells overexpressing FAM153B showed the repression of proliferation capacity more obviously. (E, F) Proliferation of MKN45 and HGC27 cells were evaluated by EdU labeling assay and showed that overexpressing FAM153B led to proliferation inhibitions of proliferation capacity.

We firstly examined endogenous expression of FAM153B in six GC cell lines mentioned in method part. Six GC cell lines expressed FAM153B in various degrees (Figure A1). The protein expression levels of FAM153B were relatively lower in MKN45 and HGC27 cells. Two cell lines were selected for further lentivirus transfection. The FAM153B overexpression group and control group were named Control and OE-FAM153B respectively. Western blotting indicated that protein expression of FAM153B was notably increased in OE-FAM153B GC cells compared with that in the control vector (Figure 6A,B). We applied MTT and EdU separately to investigate the effect of FAM153B on cell proliferation. Different degrees of cell proliferation inhibition were observed in MKN45 cell lines at 48 and 72 h, HGC27 cell lines at 24, 48 and 72 h (Figure 6C,D). Next, the results of EdU staining showed that the proliferation of MKN45 and HGC27 cells was moderately reduced in OE-FAM153B group as compared with vector group (Figure 6E,F). In summary, these results suggested that the overexpression of FAM153B could attenuate GC cell proliferation.

4. Discussion

To our knowledge, this is the first study to explore the potential effects of FAM153B in GC. This study revealed that FAM153B was downregulated in GC tissues, indicating that FAM153B has a potential impact on GC. We confirmed the low expression of FAM153B in GC via public database analysis. This observation was further validated by an IHC analysis in our independent cohort. The clinical importance of FAM153B in GC is highlighted by the correlation of lower FAM153B with adverse prognosis. To confirm the clinical relevance of FAM153B, we analyzed the relationship between FAM153B expression and clinical parameters of GC patients. The fact that a statistically significant difference between both FAM153B-low and -high group further supports its potential clinical relevance. Although the groups differed in several clinical variables, including age, gender, stage, Helicobacter pylori, the treatments, etc., these factors did not exhibit statistically significant differences. These results hint at more favorable aspects of prognosis prediction.

The pathological function of FAM153B is rarely explored in different diseases. Yet, until now only Ferdouse et al. have reported the preliminary mechanism in COPD [8]. The biological role of FAM153B in GC have not been studied. Therefore, FAM153B was selected and underwent evaluation of identifying its possible mechanism in GC. We sought its clinical significance and speculated that FAM153B has a function as the tumor suppressor gene for GC. Gene co-expression network analysis is commonly used to investigate the genes of unknown function and understand its interactions. Recent advancement in transcriptome contributed to the construction of these networks, and further infer the functions of genes. To further investigate the underlying function of FAM153B in GC, we performed the co-expression network analysis and enrichment analysis. Our results revealed enrichment of these co-expressed genes of FAM153B in primary pathways related to DNA replication. DNA replication is the fundamental physiological process that ensure accurate transfer of genetic information [9]. Dysregulation of DNA replication could cause genome instability, which was considered as a hallmark of cancer [10]. Previous studies indicated that DNA replication perturbations could have influences on the replicative stress, and been induced at the early steps of cancer development [11]. This stress could not only trigger genome instability, but also cause chromosomal instability that further generate intratumoral heterogeneity in response to external pressures [11]. Accumulating evidences suggest that several cancer cells, including gastric cancer, rely on DNA replication stress related pathways for promoting its proliferation [12–15]. After the transfection of FAM153B to MKN45 and HGC27

cell lines, we found cell proliferation suppression in the two cell lines. Edu proliferation assay could directly and accurately detect the synthesis of DNA. Therefore, we demonstrated that FAM153B was associated with DNA replication [16]. However, the specific molecular mechanism deserves further exploration.

As we all known, endogenous processes such as DNA replication could affect genome instability, thus interfering with the immunogenicity [17]. Immune cell infiltration was closely related to the immunogenicity. Growing evidences have demonstrated that the immune cell infiltration affects cancer cell viability and is associated with disease prognosis [18,19]. In the solid tumor microenvironment, the presence of dynamic tumor-immune cell interaction causes the release of cytokines and growth factors [20]. Such crosstalk could create the necessary conditions for tumor growth and metastasis [20]. Previous study suggested that Th2-like cells could orchestrate malignant phenotypes of melanoma and colorectal cancer, and might contribute to the pro-tumorigenic environment [21]. A recent study by Lan et al. found that high levels of Th2 cells are correlated with aggressive characteristics in breast cancer, especially ER-positive breast cancer [22]. The results from Schreiber S. et al elaborate unique correlations between Th2 cells and the tumor microenvironment, thereby revealing novel avenues to immunotherapies [23]. Besides, Th2 cell-related pathway has been proven to contribute to H. pylori-associated gastric tumourigenesis or intestinal metaplasia [24]. Therefore, Th2 cell might participate in the carcinogenesis process. In this study, we uncovered a negative correlation between Th2 cell and the expression level of FAM153B. Besides, our results indicate that overexpression of FAM153B could inhibit the proliferation of gastric cancer cells. In this process, Th2 cell, as primary immune cell, might be regulated by FAM153B. However, further studies are needed to confirm this inference.

Although FAM153B might act as a tumor suppressor gene in GC, there are certain limitations in this study. First, we only performed in vitro experiments to explore the potential function of FAM153B. In vivo studies were not conducted accordingly. Second, a limited number of clinical specimens were available in this study. Strict inclusion and exclusion criteria offered advantages to the collection of samples. However, future studies need to collect more samples for the exploration of correlation between FAM153B expression and relevant clinical variables, including prognostic features. Third, specific mechanisms of DNA replication and immune cell infiltration on FAM153B remain to be fully elucidated. As we now know, the occurrence and development of GC is a complex biological process involving multiple factors, and there exists various regulatory mechanisms. Therefore, further studies are warranted to provide the detailed analysis of FAM153B, to explore the underlying regulatory mechanism in tumorigenesis of GC.

5. Conclusions

In summary, this study demonstrated that FAM153B might serve as an effective prognostic biomarker and function as a tumor suppressor gene in GC.

Acknowledgments

This study was supported by the Natural Science Foundation of Gansu province (20JR10RA685, 21JR11RA099, 21JR1RA117) and the Foundation of The First Hospital of Lanzhou University (ldyyyn2018-38, ldyyyn2020-95).

Conflict of interest

The authors report no conflicts of interest in this work.

References

1. H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: A Cancer J. Clin.*, **71** (2021), 209–249. https://doi.org/10.3322/caac.21660
2. R. E. Sexton, M. N. Al Hallak, M. Diab, A. S. Azmi, Gastric cancer: a comprehensive review of current and future treatment strategies, *Cancer Metastasis Rev.*, **39** (2020), 1179–1203. https://doi.org/10.1007/s10555-020-09925-3
3. M. Banks, D. Graham, M. Jansen, T. Gotoda, S. Coda, M. di Pietro, et al., British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma, *BMJ*, **68** (2019),1545–1575. https://doi.org/10.1136/gutjnl-2018-318126
4. A. Zayac, K. Almhanna, Esophageal, gastric cancer and immunotherapy: small steps in the right direction, *Transl. Gastroenterol. Hepatol.*, **5** (2020), 9. https://doi.org/10.21037/tgh.2019.09.05
5. A. P. Thrift, H. B. El-Serag, Burden of gastric cancer, *Clin. Gastroenterol. Hepatol.*, **18** (2020), 534–542. https://doi.org/10.1016/j.cgh.2019.07.045
6. M. Cao, H. Li, D. Sun, W. Chen, Cancer burden of major cancers in China: a need for sustainable actions, *Cancer Commun.*, **40** (2020), 205–210. https://doi.org/10.1002/cac2.12025
7. T. Matsuoka, M. Yashiro, Biomarkers of gastric cancer: current topics and future perspective, *World J. Gastroenterol.*, **24** (2018), 2818–2832. https://doi.org/10.3748/wjg.v24.i26.2818
8. B. Ferdouse, I. Ruczinski, S. Li, E. Silverman, M. Cho, D. Lynch, et al., Identifying a deletion affecting total lung capacity among subjects in the COPDGene study cohort, *Genet Epidemiol*, **1** (2016), 81–88. https://doi.org/10.1002/gepi.21943
9. M. Macheret, T. D. Halazonetis, DNA replication stress as a hallmark of cancer, *Annu. Rev. Pathol.: Pathol. Mech. Dis.*, **10** (2015), 425–448. https://doi.org/10.1146/annurev-pathol-012414-040424
10. H. Zhu, U. Swami, R. Preet, J. Zhang, Harnessing DNA replication stress for novel cancer therapy, *Genes*, **11** (2020), 990. https://doi.org/10.3390/genes11090990
11. H. Kitao, M. Iimori, Y. Kataoka, T. Wakasa, E. Tokunaga, H. Saeki, et al., DNA replication stress and cancer chemotherapy, *Cancer Sci.*, **109** (2018), 264–271. https://doi.org/10.1111/cas.13455
12. J. Shi, X. Zhang, J. Li, W. Huang, Y. Wang, Y. Wang, et al., MTA2 sensitizes gastric cancer cells to PARP inhibition by induction of DNA replication stress, *Transl. Oncol.*, **14** (2021), 101167. https://doi.org/10.1016/j.tranon.2021.101167

13. M. Bauer, Z. Nascakova, A. I. Mihai, P. F. Cheng, M. P. Levesque, S. Lampart, et al., The ALPK1/TIFA/NF-κB axis links a bacterial carcinogen to R-loop-induced replication stress, *Nat. Commun.*, **11** (2020), 5117. https://doi.org/10.1038/s41467-020-18857-z
14. N. S. Sethi, O. Kikuchi, G. N. Duronio, M. D. Stachler, J. M. McFarland, R. Ferrer-Luna, et al., Early TP53 alterations engage environmental exposures to promote gastric premalignancy in an integrative mouse model, *Nat. Genet.*, **52** (2020), 219–230. https://doi.org/10.1038/s41588-019-0574-9
15. J. Rozacky, A. A. Nemec, J. B. Sweasy, D. Kidane, Gastric cancer associated variant of DNA polymerase beta (Leu22Pro) promotes DNA replication associated double strand breaks, *Oncotarget*, **6** (2015), 24474–24487. https://doi.org/10.18632/oncotarget.4426
16. H. Hua, S. E. Kearsey, Monitoring DNA replication in fission yeast by incorporation of 5-ethynyl-2'-deoxyuridine, *Nucleic Acids Res.*, **39** (2011), e60. https://doi.org/10.1093/nar/gkr063
17. E. R. Mardis, Neoantigens and genome instability: impact on immunogenomic phenotypes and immunotherapy response, *Genome Med.*, **11** (2019), 71. https://doi.org/10.1186/s13073-019-0684-0
18. D. Bruni, H. K. Angell, J. Galon, The immune contexture and immunoscore in cancer prognosis and therapeutic efficacy, *Nat. Rev. Cancer*, **20** (2020), 662–680. https://doi.org/10.1038/s41568-020-0285-7
19. W. H. Fridman, L. Zitvogel, C. Sautès-Fridman, G. Kroemer, The immune contexture in cancer prognosis and treatment, *Nat. Rev. Clin. Oncol.*, **14** (2017), 717–734. https://doi.org/10.1038/nrclinonc.2017.101
20. S. Y. Gun, S. W. L. Lee, J. L. Sieow, S. C. Wong, Targeting immune cells for cancer therapy, *Redox Biol.*, **25** (2019), 101174. https://doi.org/10.1016/j.redox.2019.101174
21. L. Halim, M. Romano, R. McGregor, I. Correa, P. Pavlidis, N. Grageda, et al., An atlas of human regulatory T helper-like cells reveals features of Th2-like tregs that support a tumorigenic environment, *Cell Rep.*, **20** (2017), 757–770. https://doi.org/10.1016/j.celrep.2017.06.079
22. L. Le, Y. Tokumaru, M. Oshi, M. Asaoka, L. Yan, I. Endo, et al., Th2 cell infiltrations predict neoadjuvant chemotherapy response of estrogen receptor-positive breast cancer, *Gland Surg.*, **10** (2021), 154–165. https://doi.org/10.21037/gs-20-571
23. S. Schreiber, C. M. Hammers, A. J. Kaasch, B. Schraven, A. Dudeck, S. Kahlfuss, Metabolic interdependency of Th2 cell-mediated type 2 immunity and the tumor microenvironment, *Front. Immunol.*, **12** (2021), 632581. https://doi.org/10.3389/fimmu.2021.632581
24. B. Marotti, A. Rocco, P. De. Colibus, D. Compare, G. de. Nucci, S. Staibano, et al., Interleukin-13 mucosal production in Helicobacter pylori-related gastric diseases, *Dig. Liver Dis.*, **40** (2008), 240–247. https://doi.org/10.1016/j.dld.2007.11.02

Appendix

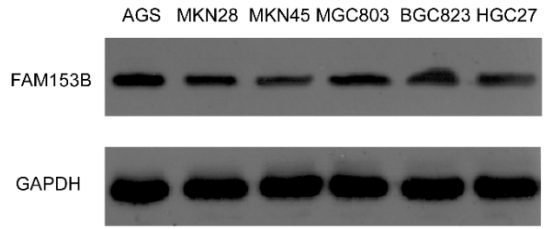

Figure A1. The expression of FAM153B in gastric cancer cell lines.

Table A1. Clinical information of patients with gastric cancer.

Id	gender	age	surgery date	fustat	futime	treatment	location	stage	T	N	M	differentiation	HER2	Hp
67186-3	male	66	2015.2.2	death	21	total gastrectomy	antrum	T4N3M0	4	3	0	poorly adenocarcinoma	+++	unknown
64549-3	male	57	2014.8.1	death	37	proximal gastrectomy	fundus	T3N1M0	3	1	0	moderately adenocarcinoma	-	（+）
64394-3	male	67	2014.8.6	death	39	proximal gastrectomy	fundus	T3N2M0	3	2	0	poorly adenocarcinoma	-	（-）
65922-2	male	71	2014.12.1	death	11	proximal gastrectomy	body	T4N2M0	4	2	0	moderately adenocarcinoma	+++	（-）
63061-3	male	72	2014.5.4	alive	60	proximal gastrectomy	fundus	T3N3M0	3	3	0	moderately adenocarcinoma	+++	unknown
62982-3	male	57	2014.4.21	death	26	proximal gastrectomy	body	T4N2M0	4	2	0	poorly adenocarcinoma		unknown

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Id	gender	age	surgery date	fustat	futime	treatment	location	stage	T	N	M	differentiation	HER2	Hp
62019-3	male	70	2014.2.27	alive	60	proximal gastrectomy	fundus	T3N0M0	3	0	0	poorly adenocarcinoma	-	unknown
69540-4	male	62	2015.7.2	death	32	proximal gastrectomy	fundus	T3N2M0	3	2	0	poorly adenocarcinoma	-	(+)
69953-1	male	70	2015.7.27	death	18	proximal gastrectomy	fundus	T4N2M0	4	2	0	poorly adenocarcinoma	-	(+)
70335-4	male	55	2015.7.10	alive	60	proximal gastrectomy	fundus	T2N1M0	2	1	0	moderately adenocarcinoma	-	(-)
68012-3	male	63	2015.3.24	death	56	proximal gastrectomy	fundus	T4N0M0	4	0	0	poorly adenocarcinoma	-	unknown
67381-3	female	51	2015.2.9	death	11	total gastrectomy	body	T4N3M1	4	3	1	poorly adenocarcinoma	-	(-)
67527-4	female	54	2015.2.25	alive	60	total gastrectomy	fundus	T3N3M0	3	3	0	poorly adenocarcinoma	-	(+)
62249-3	male	64	2014.3.5	death	15	total gastrectomy	body	T2N0M0	2	0	0	poorly adenocarcinoma	-	(-)
66530-3	male	54	2015.1.27	death	10	total gastrectomy	antrum	T3N1M0	3	1	0	moderately adenocarcinoma	-	(-)
69117-3	female	59	2015.6.4	death	2	total gastrectomy	body	T3N3M0	3	3	0	moderately adenocarcinoma	-	(+)
64747-3	male	60	2014.8.13	death	47	total gastrectomy	body	T3N2M0	3	2	0	poorly adenocarcinoma	-	(+)
69928-1	female	53	2015.7.24	alive	60	total gastrectomy	body	T4N3M0	4	3	0	moderately adenocarcinoma	-	unknown
66999-3	male	50	2015.1.24	death	33	total gastrectomy	fundus	T3N3M0	3	3	0	poorly adenocarcinoma	-	unknown

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Id	gender	age	surgery date	fustat	futime	treatment	location	stage	T	N	M	differentiation	HER2	Hp
63356-3	male	64	2014.5.14	death	10	distal gastrectomy	antrum	T4N3M1	4	3	1	mucinous adenocarcinoma	-	(-)
67039-2	male	58	2015.1.26	alive	60	distal gastrectomy	antrum	T3N1M0	3	1	0	moderately adenocarcinoma	-	(+)
67080-3	male	62	2015.1.27	death	10	distal gastrectomy	antrum	T4N3M1	4	3	1	moderately adenocarcinoma	-	(-)
62546-3	female	62	2014.3.21	alive	60	distal gastrectomy	antrum	T2N2M0	2	2	0	moderately adenocarcinoma	-	(+)
61283-4	female	65	2014.1.8	death	30	distal gastrectomy	antrum	T4aN0M0	4a	0	0	moderately adenocarcinoma	+++	(-)
66364-2	female	59	2014.12.15	death	38	distal gastrectomy	antrum	T2N3M0	2	3	0	moderately adenocarcinoma	-	(+)
63128-3	male	47	2014.4.29	alive	60	distal gastrectomy	antrum	T3N0M0	3	0	0	moderately adenocarcinoma	-	unknown
61155-3	male	42	2013.12.31	death	36	distal gastrectomy	antrum	T3N3M1	3	3	1	poorly adenocarcinoma	-	(+)
62927-3	male	54	2014.4.17	death	58	distal gastrectomy	antrum	T2N0M0	2	0	0	moderately adenocarcinoma	-	(+)
66174-3	male	56	2014.12.2	alive	60	distal gastrectomy	antrum	T1N1M0	1	1	0	highly adenocarcinoma	-	(+)
64144-3	male	67	2014.7.10	death	25	distal gastrectomy	antrum	T3N3M1	3	3	1	poorly adenocarcinoma	-	(+)
65581-3	male	68	2014.10.15	death	31	distal gastrectomy	antrum	T3N3M0	3	3	0	poorly adenocarcinoma	-	(+)
65731-3	male	69	2014.10.28	death	23	distal gastrectomy	antrum	T4N3M0	4	3	0	moderately adenocarcinoma	+++	(+)

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Id	gender	age	surgery date	fustat	futime	treatment	location	stage	T	N	M	differentiation	HER2	Hp
63432-3	male	63	2014.5.21	death	40	distal gastrectomy	antrum	T3N0M0	3	0	0	mucinous adenocarcinoma	+++	(+)
61668-3	male	53	2014.1.27	death	16	distal gastrectomy	antrum	T3N0M0	3	0	0	poorly adenocarcinoma	-	unknown
61817-3	male	48	2014.2.13	alive	60	distal gastrectomy	antrum	T2N1M0	2	1	0	poorly adenocarcinoma	-	(-)
62082-3	male	54	2014.2.27	alive	60	distal gastrectomy	antrum	T2N1M0	2	1	0	poorly adenocarcinoma	-	(+)
62760-3	male	57	2014.4.8	death	42	distal gastrectomy	antrum	T3N3M0	3	3	0	poorly adenocarcinoma	-	(+)
63163-3	female	46	2014.5.4	alive	60	distal gastrectomy	antrum	T2N0M0	2	0	0	poorly adenocarcinoma	-	unknown
63321-2	female	63	2014.5.21	death	28	distal gastrectomy	antrum	T4N1M0	4	1	0	moderately adenocarcinoma	-	(-)
63489-3	male	61	2014.5.26	alive	60	distal gastrectomy	antrum	T3N2M0	3	2	0	moderately adenocarcinoma	-	unknown
64067-4	male	40	2014.7.4	death	27	distal gastrectomy	antrum	T3N2M0	3	2	0	poorly adenocarcinoma	-	unknown
64528-2	female	56	2014.7.31	death	20	distal gastrectomy	antrum	T4N3M1	4	3	1	poorly adenocarcinoma	-	(+)
65313-3	male	73	2014.9.19	alive	60	distal gastrectomy	antrum	T3N0M0	3	0	0	poorly adenocarcinoma	-	(+)
65405-3	male	51	2014.9.29	death	39	distal gastrectomy	antrum	T3N3M0	3	3	0	poorly adenocarcinoma	-	(+)
66135-3	male	43	2014.11.28	death	35	distal gastrectomy	antrum	T3N0M0	3	0	0	poorly adenocarcinoma	-	(-)

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Id	gender	age	surgery date	fustat	futime	treatment	location	stage	T	N	M	differentiation	HER2	Hp
66541-3	female	41	2014.12.26	alive	60	distal gastrectomy	antrum	T1N1M0	1	1	0	poorly adenocarcinoma	-	(+)
66972-3	female	67	2015.1.23	death	41	distal gastrectomy	body	T3N1M0	3	1	0	mucinous adenocarcinoma	-	(-)
63528-3	male	58	2014.5.28	alive	60	distal gastrectomy	antrum	T2N2M0	2	2	0	poorly adenocarcinoma	-	(+)
66457-3	male	40	2015.1.26	alive	60	distal gastrectomy	antrum	T1N1M0	1	1	0	moderately adenocarcinoma	-	(+)
69284-3	female	60	2015.6.15	alive	60	distal gastrectomy	antrum	T1N0M0	1	0	0	moderately adenocarcinoma	-	(-)
70634-3	female	68	2015.9.10	death	30	distal gastrectomy	antrum	T3N0M0	3	0	0	moderately adenocarcinoma	-	(+)
70708-3	female	68	2015.9.16	death	50	distal gastrectomy	antrum	T1N2M0	1	2	0	highlyadenocarcinoma	-	(-)
68762-3	male	60	2015.5.13	death	58	distal gastrectomy	antrum	T1NOM0	1	0	0	highlyadenocarcinoma	-	(-)
70411-3	female	69	2015.8.24	death	31	distal gastrectomy	antrum	T3N2M0	3	2	0	moderately adenocarcinoma	-	(-)
68634-2	male	45	2015.5.5	alive	60	distal gastrectomy	antrum	T1N0M0	1	0	0	moderately adenocarcinoma	-	(-)
68054-3	male	58	2015.3.26	death	9	distal gastrectomy	antrum	T2N3M1	2	3	1	poorly adenocarcinoma	-	(+)
68131-3	female	76	2015.4.1	death	31	distal gastrectomy	antrum	T1bN2M0	1	2	0	poorly adenocarcinoma	-	(-)
68354-3	female	60	2015.4.15	death	13	distal gastrectomy	antrum	T4N3M1	4	3	1	poorly adenocarcinoma	-	(-)

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Id	gender	age	surgery date	fustat	futime	treatment	location	stage	T	N	M	differentiation	HER2	Hp
69285-3	male	57	2015.6.15	death	38	distal gastrectomy	antrum	T3N1M0	3	1	0	poorly adenocarcinoma	-	(+)
70841-2	male	62	2015.9.24	alive	60	distal gastrectomy	antrum	T3N0M0	3	0	0	moderately adenocarcinoma	-	(-)
70982-3	male	49	2015.10.12	alive	60	distal gastrectomy	body	T4N2M0	4	2	0	moderately adenocarcinoma	-	(+)
71194-3	male	60	2015.10.29	alive	60	distal gastrectomy	antrum	T2N2M0	2	2	0	poorly adenocarcinoma	-	(-)
69050-4	male	65	2015.5.24	alive	60	distal gastrectomy	antrum	T2N0M0	2	0	0	poorly adenocarcinoma	-	(+)
71357-3	male	78	2015.11.11	death	13	distal gastrectomy	antrum	T4N3M0	4	3	0	moderately adenocarcinoma		(-)
68518-3	male	48	2015.4.23	death	45	distal gastrectomy	antrum	T3N3M0	3	3	0	mucinous adenocarcinoma	-	unknown