

Prognostic Significance and Immune Microenvironment Correlates of coagulation factor 2 receptor in Gastric Cancer and Retrospective Verification

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Abstract

Objective: To assess the prognostic role of coagulation factor 2 receptor (F2R) in gastric cancer (GC).

Method: In this retrospective study, data from gastric cancer patients treated with radical gastrectomy between March and August 2023 in Xishan People's Hospital were retrieved and analyzed from July to August 2024. Public databases (The Cancer Genome Atlas, Gene Expression Omnibus, University of California Santa Cruz Xena, and Tumour Immune Estimation Resource 2.0) were used to assess F2R expression, methylation, and immune infiltration. Prognostic factors were evaluated using Kaplan Meier analysis, Cox regression, nomograms, and MethSurv. Gene Ontology and Kyoto Encyclopaedia of Genes and Genomes enrichment analyses were performed on genes co-expressed with F2R.

Results: F2R was significantly upregulated in gastric cancer tissues ($p < 0.001$) and correlated with higher histological grade and advanced clinical stage ($p < 0.001$). High expression of F2R independently predicted poorer overall survival (hazard ratio: 2.004; 95% confidence interval: 1.069-3.755). Low promoter methylation was associated with worse prognosis ($p < 0.05$). F2R expression showed positive correlations with cluster of differentiation 8-positive (CD8+) T cells, fibroblasts, and macrophages. Single-cell analysis confirmed expression of F2R in fibroblasts, CD8+ T cells, and plasma cells. Pathway enrichment revealed involvement in cancer related signalling, including Phosphatidylinositol 3-kinase-Protein kinase B, Mitogen-Activated Protein Kinase, extracellular matrix receptor interaction, and focal adhesion pathways. In silico screening identified vorapaxar and thrombin as potential drugs targeting F2R.

Conclusions: Coagulation factor 2 receptor may be an independent prognostic biomarker for gastric cancer and may correlate with immune infiltration.

Keywords: Coagulation factor 2 receptor, F2R, Gastric cancer, Prognosis, Immune infiltration, Functional enrichment.

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Introduction

Gastric cancer (GC) is a globally common malignant tumour of the digestive system. According to estimates¹ compiled by the International Agency for Research on Cancer, 1.09 million new GC cases occurred globally in 2020, resulting in approximately 769,000 deaths. GC ranks fifth in both the incidence and mortality rates among all cancers worldwide. The incidence rate in male is about twice that in female, and the incidence rates are significantly higher in Asian populations.² Key risk factors for GC include *helicobacter (H.) pylori* infection, alcohol

consumption, smoking and salt-cured foods,³ low intake of fruits, and high intake of processed and barbecued meats. Due to the lack of specific symptoms in early GC patients, most cases are diagnosed at an advanced stage.⁴ Although the promotion of early screening technologies and improved treatment methods, such as surgery, radiotherapy and chemotherapy, can somewhat improve patient survival, the 5-year survival rate for GC is still not high due to tumour recurrence, adverse treatment reactions and chemotherapy resistance.⁵⁻⁸ In addition, owing to the high infection rate of *H. pylori* and the trend of aging population, the disease burden of GC is expected to remain severe. Therefore, GC poses a serious threat to human health, causing significant physical harm, mental stress and economic burden to individuals, families and social health systems, making it a critical public health problem.

The pathogenesis of GC is complex, involving individual genetic variations, immune responses, environmental factors, microbial infections, age, gender, diet and lifestyle. Delving into the molecular mechanisms underlying GC onset and progression, and identifying sensitive molecular diagnostic markers and therapeutic targets, is essential to improve patient outcomes. Proteinase-activated receptors

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(PARs), members of the G protein-coupled receptor family, are activated by serine proteases, such as thrombin. Among them, PAR-1 (gene symbol: F2R) is the canonical high-affinity thrombin receptor. F2R is widely expressed in normal cells, such as epithelial cells, endothelial cells, fibroblasts, immune cells and neurons,⁹ with expression potentially elevated in certain pathological conditions. Research has shown that activated coagulation factor 2 receptor (F2R) participates in both physiological and pathological processes, including inflammation, coagulation response, thrombosis and tumour angiogenesis.⁹ Furthermore, F2R is highly expressed across many tumour cell lines, for example, gastric cancer,¹⁰ breast cancer,¹¹ pancreatic cancer,¹² liver cancer,¹³ colorectal cancer¹⁴ and glioma.¹⁵ Additionally, a significant correlation exists between tumour coagulation and the tumour microenvironment (TME).¹⁶ Coagulation score, constructed from coagulation-related genes, is closely associated with tumour invasiveness, angiogenesis, genomic stability, proliferation and metastatic potential.¹⁷ However, the prognostic value of F2R expression in GC patients and its correlation with immune infiltration are yet to be elucidated.

Although studies have suggested that F2R is highly expressed in various tumours,¹⁰⁻¹⁵ its expression pattern in GC, its relationship with clinical staging, whether it is an independent prognostic factor, and its specific role in the tumour immune microenvironment are still unclear. The current study was planned to fill the gap in literature by assessing the prognostic role of F2R in GC.

Materials and Methods

The retrospective study was conducted at Xishan People's Hospital and Xuzhou Medical University from July 2024 to August 2024 and comprised pathological samples of patients having undergone radical gastrectomy between March 2023 and August 2023 in the General Surgery Department of Xishan People's Hospital, China.

Data were collected from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>),¹⁸ Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>),¹⁹ Tumour Immune Estimation Resource-2.0 (TIMER2.0) database (<http://timer.cis-trome.org/>)²⁰ and the University of California, Santa Cruz (UCSC) Xena (<https://xena.ucsc.edu/>) database.²¹ The gene expression data and related clinical information were obtained from the TCGA and GEO databases, which included 375 GC tumour tissues and 32 normal tissues from TCGA, as well as 300 tumour tissues and 100 normal tissues from GEO (GSE63089, etc.). Normalization and Log2 conversion of gene expression data were performed using the

Sangerbox3 tool (<http://sangerbox.com>).²² After cleaning the data and excluding samples with missing clinicopathological information or follow-up duration, the original gene expression profile data was organised into a gene expression matrix file with the Sangerbox3 tool.

The Wilcoxon signed-rank test was used to analyse F2R expression differences in GC and tumour-adjacent tissue samples, employing adjusted $p < 0.05$ and fold change > 2 as the screening criteria. Paired t-tests assessed the differential expression of F2R in GC and corresponding adjacent tissue samples. If the data did not meet the assumptions of normality, non-parametric tests were applied. Finally, differences in the expression of F2R in tumour samples of different stages were analysed using analysis of variance (ANOVA).

Based on curated clinical data, univariate Cox regression and multivariate Cox regression analysis were used to explore the significance of age, gender, clinical stage, pathological grade and F2R expression level in the prognosis of GC patients, and R package forestploter version 1.1.3 was used for visual analysis. R package maxstat version 0.7-26 was used to calculate the optimal cut-off value of F2R expression. The minimum sample size of each group was specified to exceed 25%, and the maximum sample size of each group was specified to be $< 75\%$. Subsequently, the final optimal cut-off value was obtained. Patients were stratified into high-expression and low-expression groups based on this cut-off value. Differences in overall survival (OS) between groups were analysed using the R package 'survival' (version 3.8-3), employing the Kaplan-Meier method for comparison. A prognostic nomogram and calibration curves were constructed using the Cox proportional hazards model via the R package 'rms' (version 6.8-0), incorporating survival time, survival status, and variables significantly associated with prognosis. Calibration curve assessment was performed at three predefined clinical time points: 1-year, 3-year and 5-year OS. Internal validation was conducted via bootstrapping, with each iteration drawing 75 samples, repeated for 5 iterations to ensure estimate stability.

Deoxyribonucleic acid (DNA) methylation levels of individual Cytosine-phosphate-guanine (CpG) sites within the F2R promoter region were obtained from the UCSC Xena database. To assess the relationship between methylation and gene expression, Pearson correlation analysis was performed between the methylation level of each CpG site and the corresponding F2R expression value from the same sample set. Statistical significance was defined as two-tailed $p < 0.05$.²³ The MethSurv website (<https://biit.cs.ut.ee/methsurv/>) was used to visualise the characteristics of methylation sites in the F2R promoter

region and the relationship between methylation levels and patient prognosis.²⁴

The TME comprises infiltrating immune cells, stromal cells, tumour cells and extracellular matrix. The TME of GC has been reported to be mainly composed of non-tumour cells, such as stromal cells and immune cells.^{16,25} The ESTIMATE algorithm (version 1.0.13) was employed to utilize transcriptomic data from cancer samples to evaluate the stromal content and immune cells in tumour tissue, and to calculate the immune score and matrix score.²⁶ TIMER2.0 was applied to evaluate the correlation between F2R expression and tumour infiltrating immune cells (TIIC) abundance, including cluster of differentiation 4 (CD4) +T cell, CD8+T cell, and macrophage. TIMER 2.0 incorporated correction for tumour purity and partial copy number variation to improve quantification accuracy of immune subsets in bulk tumour tissue. ESTIMATE and TIMER2.0 were used because of their established performance in pan-cancer analyses, direct purity estimation, and reduced dependence on batch-specific normalisation. For single-cell datasets, Tumour Immune Single-Cell Hub 2 (TISCH2) provides dataset information, cell type annotation and expression visualisation.²⁷

LinkedOmics (www.linkedomics.org) online tool was employed to screen for F2R co-expressed genes, with a set standard of $r=0.3$ and $p<0.05$ (Figure 1 A-C).²⁸ The ClusterProfiler package in R was used for Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the co-expressed genes of F2R. Gene enrichment analysis of F2R was performed by Gene Set Enrichment Analysis (GSEA) 4.1.0 with KEGG gene sets, and 1,000 random combinations were performed by default weighted enrichment statistics. The gene sets obtained with $p<0.05$ and false discovery rate (FDR) <0.05 were considered significantly enriched gene sets. Finally, potential drugs that interact with F2R were analysed using the Tumour-Immune System Interaction Database (TISIDB (<http://cis.hku.hk/TISIDB/>)) online tool.²⁹

The present study was granted approval by the ethics committee of Xishan People's Hospital. Pathological samples of patients undergoing radical gastrectomy were randomly selected for immunohistochemical (IHC) verification.

IHC staining was performed using the streptavidin-peroxidase method with a commercial detection kit (KIT-5010, Maixin.Bio, Fuzhou, China). Briefly, tissue sections were deparaffinised, rehydrated and subjected to antigen retrieval by high-pressure heating in citrate buffer (potential of Hydrogen [pH] 6.0). After washing,

endogenous peroxidase activity was blocked by incubating with solution A for 10 min at room temperature. The sections were then washed with Phosphate-Buffered Saline (PBS) and incubated with solution B for 10 min at room temperature. The primary antibody against F2R (rabbit polyclonal, Cat# ab117749, Abcam, Cambridge, UK) was diluted at 1:200, applied to the sections, and incubated at 4°C overnight. After thorough PBS washes, solutions C and D were sequentially applied, each for 10 min at room temperature. Colour development was carried out using 3,3'-diaminobenzidine (DAB), followed by counterstaining with haematoxylin, dehydration and mounting. Each tissue section was independently evaluated by two experienced pathologists blinded to the clinical data. A semi-quantitative scoring system was applied based on the percentage of positive tumour cells and staining intensity. Ten representative high-power fields (HPFs; $\times 400$ magnification) were randomly selected and analysed under a light microscope, and the number of positive cells was counted among approximately 100 consecutive cells per field to estimate the positivity rate. The proportion of positive cells was then scored as: 0 = $<10\%$, 1 = $10\sim 25\%$, 2 = $26\sim 50\%$, 3 = $51\sim 75\%$ and 4 = $76\sim 100\%$. Staining intensity was graded as 0 = negative, 1 = weak (light brown), 2 = moderate (brown), and 3 = strong (dark brown). The final IHC score was calculated by multiplying the proportion and intensity scores, resulting in a composite score ranging 0-12.³⁰ To evaluate the agreement between the two independent pathologists, Cohen's kappa statistic was calculated based on the categorical IHC scores. Discrepancies between the evaluators were resolved through discussion.

Data was analysed using R version 4.4.0. Prior to analysis, the normality of continuous variables was assessed using the Shapiro-Wilk test. For data that met the normality assumption, parametric tests were applied, including student's t test (paired or unpaired), which was used for comparisons between two groups, and one way ANOVA, which was used for comparisons among more than two groups. For data that did not meet the normality assumption, non parametric tests were employed, including the Wilcoxon signed rank test, which was used for two-group comparisons. Kaplan-Meier method was used to evaluate GC survival status. Univariate and multivariate Cox regression models were used to analyse the influencing factors of patient survival. The proportional hazards assumption was tested using Schoenfeld residuals. Spearman correlation coefficient was used to evaluate the correlation. The threshold for statistical significance was set at $p<0.05$.

Results

Of the 12 patients, 9(75%) were male and 3(25%) were females. There were 8(66.7%) patients aged >60 years, while 4(33.3%) were aged <60 years (Table). F2R expression was significantly elevated in GC tissues compared to adjacent normal tissues in both the TCGA and GEO (GSE66229) cohorts ($p<0.001$) (Figure 1 A-D). IHC analysis confirmed higher F2R protein levels in the tumours (Figure 1 E-G). F2R expression was positively associated with aggressive disease features, like it was higher in poorly differentiated (G3) tumours than in well/moderately differentiated (G1/G2) tumours ($p<0.01$) (Fig. 1H) and varied significantly across pathological stages ($p=0.013$), with a rising trend from stage I to III, followed by a decline in stage IV (Figure 1I).

In univariate and multivariate Cox regression analyses, high F2R expression was identified as an independent

Table: Clinical characteristics of the patients (n=12).

Characteristic	n(%)
Gender	
Female	3(25.0)
Male	9(75.0)
Age (years)	
≤60	4(33.33)
>60	8(66.67)
T stage	
T1	2(16.67)
T2	1(8.33)
T3	2(16.67)
T4	7(58.33)
N stage	
N0	6(50.0)
N1	2(16.67)
N2	3(25.0)
N3	1(8.33)
M stage	
M0	12(100)
M1	0(0)

T: Tumour, N: Node, M: Metastasis.

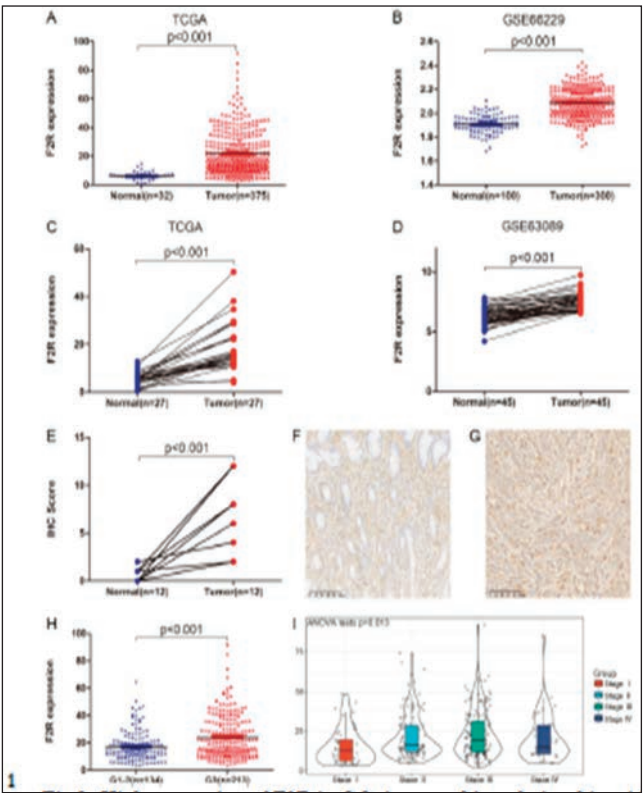


Figure-1: High expression of coagulation factor 2 receptor (F2R) in gastric cancer (GC) tissue and its relationship with clinical stage and histological grade. (A-B) The expression of F2R increased in GC tissue and adjacent normal gastric tissue based on The Cancer Genome Atlas (TCGA) and GSE66229 dataset, respectively. (C-D) The expression of F2R increased in paired GC tissue and matched adjacent normal gastric tissue in the TCGA and GEO dataset. (E) Scoring of F2R in 12 GC and 12 normal gastric tissue as detected by immunohistochemistry (IHC). The expression of F2R in the tumour tissue (G) and adjacent tissue (F) by IHC (x20). The histological grade (H) and clinical stage (I) of GC were correlated with the expression of F2R.

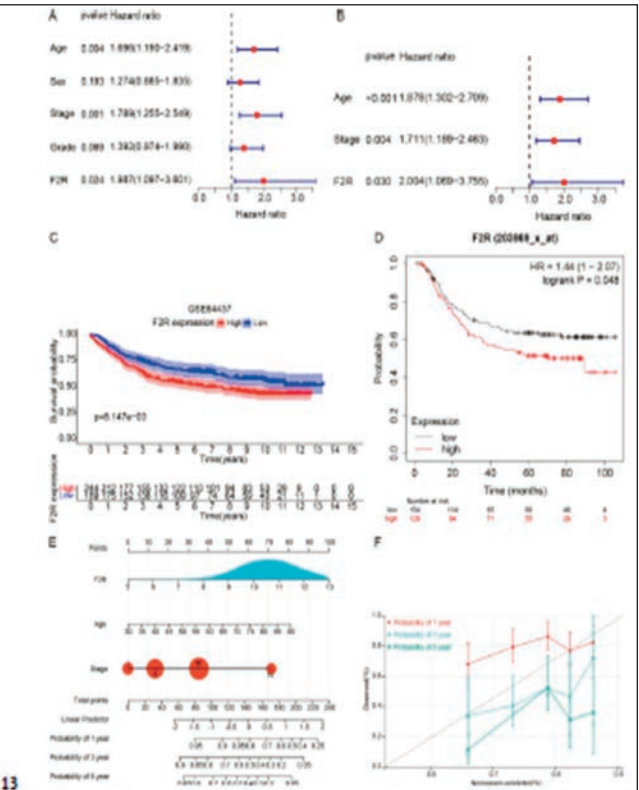


Figure-2: Univariate and multivariate Cox regression analysis of survival in coagulation factor 2 receptor (F2R) high expression group and low expression group of gastric cancer (GC) and nomogram predicting. (A) Univariate Cox regression. (B) Multivariate Cox regression. (C-D) Kaplan-Meier curves of survival probability in patients with GC according to the high or low expression level of F2R. (E) Nomogram predicting probability in GC. (F) The nomogram prediction calibration curve.

prognostic factor significantly associated with poorer OS (hazard ratio [HR]: 2.004; 95% confidence interval [CI]: 1.069-3.755; $p < 0.05$), along with older age and advanced stage (Figure 2 A-B). Consistently, Kaplan-Meier survival curves from external datasets showed significantly shorter survival for patients with high F2R expression ($p < 0.05$) (Figure 2 C-D). A prognostic nomogram integrating these variables demonstrated moderate predictive accuracy (C-index: 0.656) (Figure 2E), with calibration curves indicating good agreement between predicted and observed 1-year, 3-year and 5-year survival (Figure 2F).

The F2R promoter region was hypomethylated in GC (Figure 3A), and low promoter methylation correlated with poorer OS (Figure 3B). Methylation levels at specific CpG sites (e.g., cg11591325, cg14773260) were inversely correlated with F2R gene expression (Figure 3C). Survival analysis of 19 promoter CpG sites identified four sites (cg24702798, cg11591325, cg14773260, cg24678700)

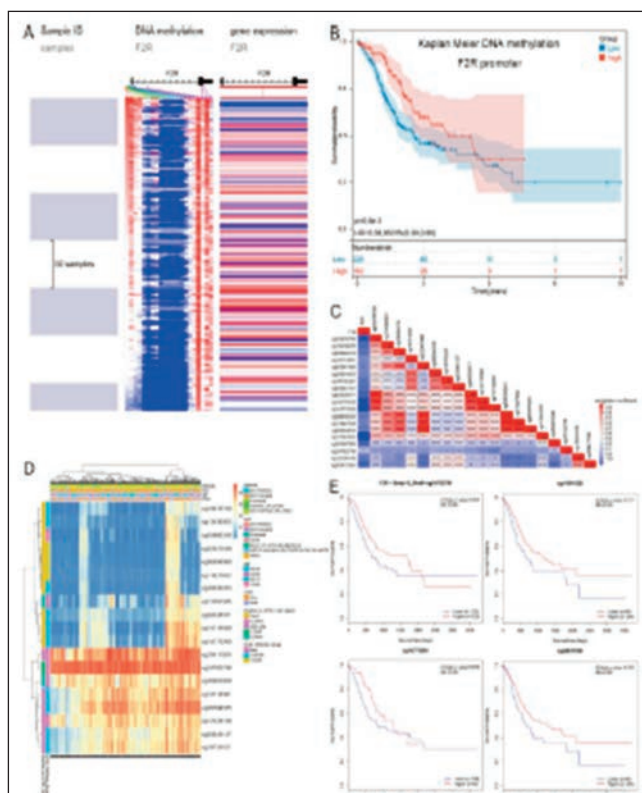


Figure-3: The deoxyribonucleic acid (DNA) hypomethylation and gene expression of coagulation factor 2 receptor (F2R) in patients with gastric cancer (GC). (A) The gene expression and methylation level of F2R (data from University of California, Santa Cruz [UCSC] Xena). (B) Kaplan-Meier curves of survival probability in patients with GC according to the high or low deoxyribonucleic acid DNA methylation level of F2R promoter. (C) Hypomethylation of F2R promoter region exhibited a negative correlation with gene expression. (D) Relationship between methylation levels of DNA sites and clinical and pathological characteristics. (E) Kaplan-Meier curves for survival probability of methylation levels in the F2R promoter region.

where hypomethylation significantly predicted worse patient prognosis ($p < 0.05$) (Figure 3 D-E).

F2R expression showed strong positive correlations with both stromal and immune scores ($r = 0.71$ and $r = 0.45$, respectively; $p < 0.001$) (Figure 4 A-B). F2R levels were

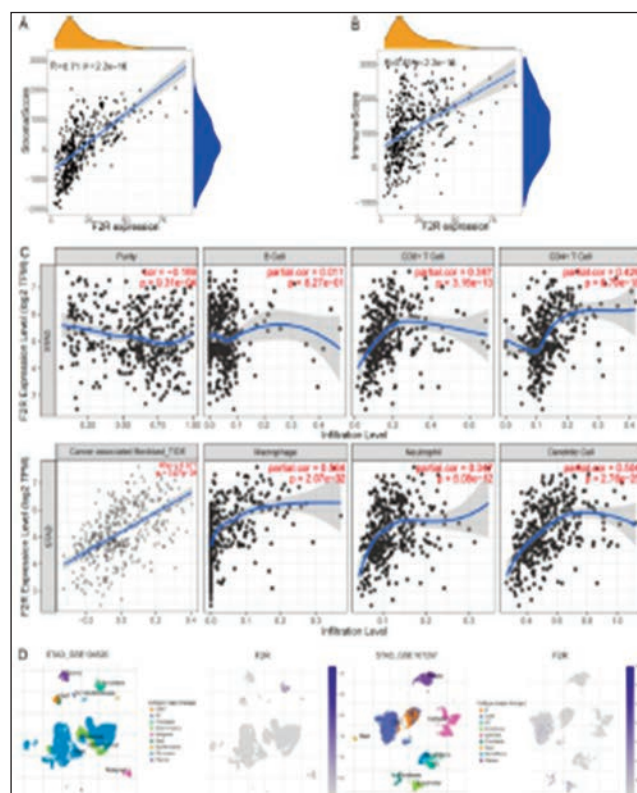


Figure-4: Effect of coagulation factor 2 receptor (F2R) on tumour microenvironment. (A) Stromal score. (B) Immune score. (C) Correlation between F2R expression and immune infiltration level. (D) Single-cell analysis of F2R.

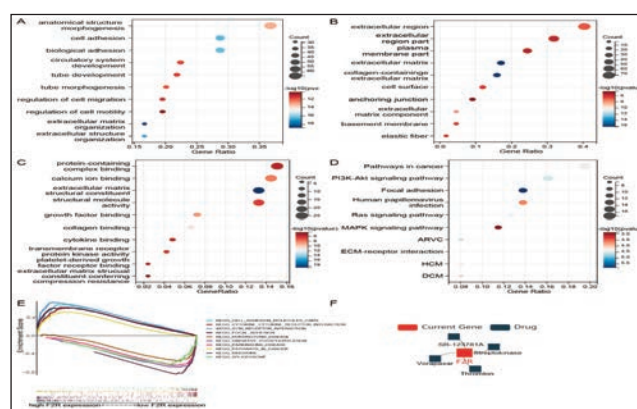


Figure-5: Functional enrichment analysis of coagulation factor 2 receptor (F2R) co-expressed genes and screening of therapeutic drugs. Gene Ontology (GO) annotation analysis: (A) biological processes, (B) cellular component, (C) molecular functions, and (D) Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis of F2R co-expressed genes. (E) Screening of therapeutic drugs for F2R.

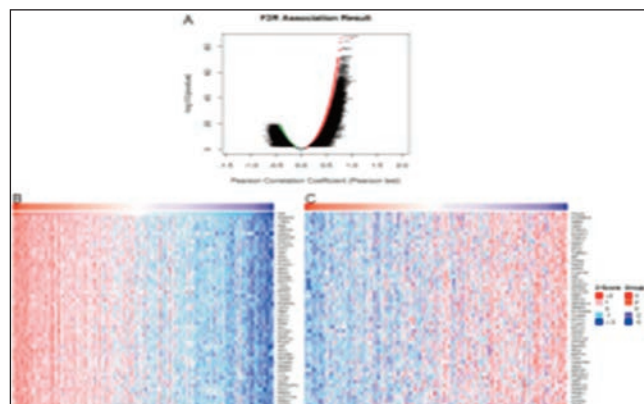


Figure Supplement 1: Genes correlated with coagulation factor 2 receptor (F2R) expression. (A) Volcano plot of genes correlated with F2R expression. (B) Heatmap of genes positively correlated with F2R. (C) Heatmap of genes negatively correlated with F2R.

positively associated with infiltration of multiple immune cell types, including CD8⁺ T cells, cancer-associated fibroblasts, macrophages and dendritic cells ($p < 0.05$) (Figure 4C). Single-cell Ribonucleic acid sequencing (RNA-seq) analysis revealed F2R expression not only in fibroblasts, but also in immune cell populations, such as CD8⁺ T cells and plasma cells (Figure 4D).

Functional enrichment analysis of F2R co-expressed genes highlighted their involvement in extracellular matrix organisation, cell adhesion and plasma membrane components (Figure 5 A-C). There was significant enrichment in cancer-related signalling, including the Phosphatidylinositol 3-kinase–Protein kinase B (PI3K-Akt) and Mitogen-Activated Protein Kinase (MAPK) pathways, focal adhesion and extracellular matrix (ECM) receptor interaction (Figure 5D). GSEA corroborated that high F2R expression was linked to activation of pathways, such as ECM-receptor interaction, focal adhesion and general cancer pathways (Figure 5E). In silico screening identified several compounds, like vorapaxar and thrombin, with documented or predicted activity against F2R as potential candidates for further investigation (Figure 5F).

Discussion

With the development of medicine and the precision of GC treatment plans, the use of targetted drugs, such as immune checkpoint inhibitors and cell immunotherapy, has enhanced the survival rate among some GC patients. However, the molecular mechanism that affect the occurrence, development and prognosis of GC are still not fully elaborated. The coagulation cascade has been demonstrated to have a close association with the progression of tumours.^{31,32} Studies have found that F2R is highly expressed in various cancers, such as GC, colorectal adenocarcinoma, colorectal adenocarcinoma and

oesophageal cancer.³³⁻³⁵ Since patients with high expression of F2R have a poorer prognosis, it is speculated that F2R may play an important role in regulating tumour progression. Especially, clinical pathological analysis can reflect tumour heterogeneity, which can provide new ideas for identifying indicators of prognosis and chemotherapy response in GC patients.³⁶

Inspired by these findings, through the correlation analysis of F2R expression and tumour pathological factors, univariate and multivariate analysis using Cox proportional risk model, and nomogram prognostic model, the current study found that F2R was an important independent prognostic factor for GC. Additionally, the methylation analysis of promoter region revealed that F2R expression significantly influences the prognosis of GC patients. Furthermore, F2R expression is associated with immune cell expression in TME and was positively correlated with CD8⁺ T cells, tumour-associated fibroblasts, macrophages and so on, which suggested that F2R may be involved in the process of immune cell infiltration in GC. Finally, the results of functional enrichment analysis showed that high expression of F2R gene could affect the occurrence and development of gastric cancer by promoting ECM receptor interaction, PI3K-Akt signalling pathway, MAPK signalling pathway and other pathways in cancer.

The role of F2R in cancer has been reported in multiple tumour types, though its functions may vary across contexts. While studies in GC, colorectal and other cancers have linked high F2R expression to tumour progression and poor prognosis, its specific role in the GC immune microenvironment remains less defined. the current findings not only confirm the prognostic value of F2R, but further reveal its association with promoter hypomethylation and specific immune cell infiltration, offering a more integrated perspective. Liu et al. established a prognostic model for GC³⁷ and Wu et al. 35 analysed the results by using the nomogram, which all confirmed that F2R could be used as a reference and potential biomarker for the prognosis of GC. The present study reported similar findings. In terms of epigenetics, it is generally believed that hypomethylation of the promoter region is associated with high gene expression. By using multiple omics analyses to elucidate the upstream mechanism of F2R expression, it was found that significant changes in the promoter or exon methylation status of F2R were detected in relation to tumour³⁸ and aging.³⁹ The current results found that DNA methylation had a significant impact on F2R expression and OS in patients with GC. Therefore, it can be considered that the regulation of F2R expression through the DNA methylation pathway affects the survival of patients. However, more studies on

the relationship between promoter methylation of F2R in GC and tumorigenesis and progression are needed to explore detailed mechanisms.

In recent years, with the development and widespread application of single-cell sequencing technology, the relationship between immune microenvironment, immune infiltration and tumour has become a research hotspot.^{40,41} However, the mechanism by which coagulation-related molecule F2R affects immune regulation in GC remains unclear. In this study, by comparing the expression of F2R in different cell populations, it was found that high expression of F2R was significantly correlated with tumour-infiltrating lymphocyte (TIL) levels (CD8+ T cells), fibroblasts and vascular endothelial cells, suggesting that F2R might be a new target for immunotherapy of GC. Liu et al.³⁷ conducted risk stratification by exploring immune genes and TME in GC and constructing an immune-related risk score (IRRS) model containing Apolipoprotein H (APOH), Ribonuclease A Family Member 2 (RNASE2), F2R, Defensin Beta 126 (DEFB126), C-X-C Motif Chemokine Ligand (CXCL6) and CXCL3 genes. Risk levels were found to be significantly associated with TILs, immune checkpoint molecular expression, and immunophenotypic score (IPS). Their findings suggested that F2R may participate in immunomodulatory mechanisms in GC. Notably, the current results suggest a potential mechanistic link: promoter hypomethylation may drive F2R overexpression, which in turn correlates with a distinct immunosuppressive microenvironment characterised by increased infiltration of CD8+ T cells, cancer-associated fibroblasts, and macrophages. This epigenetic-immune axis positions F2R as a potential modulator of both tumour aggressiveness and immune contexture in GC.

To further elucidate the molecular function of F2R in GC, GO, KEGG and GSEA analyses were performed in the current study. It was found that the cancer pathway, PI3K-Akt signalling pathway, Ras signalling pathway, MAPK signalling pathway, ECM receptor interaction and other signalling pathways exhibited significant difference. From the perspective of molecular mechanism, these results also suggested that F2R could be associated with tumour progression and affect patient prognosis through the above signalling pathways. In addition, F2R was closely related to tumour immunity. Tsai et al. found by sequencing that F2R participated in the regulation of PI3K-Akt signalling pathway and was related to cell proliferation and apoptosis.⁴² In order to identify key genes and pathways involved in gastric adenocarcinoma, Zhang et al.⁴³ analysed the prognosis of patients by transcriptome sequencing combined with survival data of TCGA database, and found 15 differentially expressed genes, including F2R, as well

as key pathways that might play important roles in the progression of gastric adenocarcinoma, for example platelet activation, cell apoptosis and cell cycle. The MAPK signalling pathway is a related pathway that regulates the cell cycle.⁴⁴ These results are consistent with the current findings.

TME and ECM are receiving increasing attention as being new research hotspots. The ECM not only provides structural support to the normal physiological activities of tissue cells, but also plays an important role in immune regulation. Meanwhile, the immune system can maintain the homeostasis of the matrix microenvironment. So, ECM and immune cells are interdependent.⁴⁵ Similarly, tumour cells and stromal cells also engage in reciprocal communication. ECM contains various secreted proteins, including cytokines, chemokines and growth factors, which play an important role in immune cell regulation. Aberrant prothrombin,⁴⁶ long-term chronic gastritis, *H. pylori* infection,⁴⁷ etc. change the chemical composition, structure and biological functions of ECM, produce cascade reactions and start immune regulation, which may play an important role in tumour progression and metastasis.⁴⁸

The current study's findings address a key research gap regarding F2R's prognostic mechanisms and immune regulatory functions in GC. However, the current study has several limitations. First, the prognostic nomogram was constructed and evaluated solely within the TCGA cohort without independent external validation or rigorous internal resampling (e.g., bootstrapping), which may affect its generalisability to other populations. Second, although IHC supported the transcriptional findings, the validation cohort was small (n=12), limiting statistical power and broader applicability. Third, the study did not evaluate whether F2R functions uniformly across different GC subtypes (e.g., intestinal vs. diffuse type), and the precise downstream targets and interacting partners of F2R in relevant signalling pathways remain to be elucidated. Fourth, while multi-database integration enhanced robustness, the findings are derived from public repositories and may be influenced by cohort-specific biases or platform effects; for instance, the non-linear change in F2R expression across stages warrants further validation. Fifth, the methylation analysis of multiple CpG sites was exploratory and did not apply multiple-testing correction, increasing the potential for false positives. Finally, the associations reported are observational and do not establish causality. Addressing these limitations in future studies will strengthen the clinical and biological relevance of F2R in GC.

Conclusions

The high expression of F2R was found associated with poor prognosis of GC, and F2R could be considered an independent risk factor. Through methylation modification and influence on immune microenvironment, F2R may be correlated with immune cell function through signalling pathways, and participate in the occurrence, progression and metastasis of GC, leading to poor prognosis of GC patients. F2R could be used as a potential novel prognostic marker and a potential therapeutic target for GC patients.

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Conflict of Interest: None.

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References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209–49. doi:10.3322/caac.21660.
- Yang WJ, Zhao HP, Yu Y, Wang JH, Guo L, Liu JY, et al. Updates on global epidemiology, risk and prognostic factors of gastric cancer. *World J Gastroenterol.* 2023;29:2452–68. doi:10.3748/wjg.v29.i16.2452.
- Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. *Gastroenterology.* 2017;153:420–9. doi:10.1053/j.gastro.2017.04.022.
- Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. *Lancet.* 2016;388:2654–64. doi:10.1016/s0140-6736(16)30354-3.
- GASTRIC Group; Paoletti X. Benefit of adjuvant chemotherapy for resectable gastric cancer. *JAMA.* 2010;303:17. doi:10.1001/jama.2010.534.
- Claassen YHM, van Amelsfoort RM, Hartgrink HH, Dikken JL, de Steur WO, van Sandick JW, et al. Effect of hospital volume with respect to performing gastric cancer resection on recurrence and survival. *Ann Surg.* 2019;270:1096–102. doi:10.1097/sla.0000000000002940.
- Chittawadagi B, Nayak SK, Ramakrishnan P, Kumar S, Cumar B, Natarajan R, et al. Laparoscopic D2 gastrectomy in advanced gastric cancer: postoperative outcomes and long-term survival analysis. *Asian J Endosc Surg.* 2021;14:707–16. doi:10.1111/ases.12926.
- Ito Y, Miyashiro I, Ishikawa T, Akazawa K, Fukui K, Katai H, et al. Determinant factors on differences in survival for gastric cancer between the United States and Japan using nationwide databases. *J Epidemiol.* 2021;31:241–8. doi:10.2188/jea.JE20190351.
- Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature.* 2000;407:258–64. doi:10.1038/35025229.
- Otsuki T, Fujimoto D, Hirono Y, Goi T, Yamaguchi A. Thrombin conducts epithelial–mesenchymal transition via protease-activated receptor-1 in human gastric cancer. *Int J Oncol.* 2014;45:2287–94. doi:10.3892/ijo.2014.2651.
- Yu M, Chen Y, Wang Z, Ding X. pHLIP(Var7)-P1AP suppresses tumor cell proliferation in MDA-MB-231 triple-negative breast cancer by targeting protease activated receptor 1. *Breast Cancer Res Treat.* 2020;180:379–84. doi:10.1007/s10549-020-05560-2.
- Arakaki AKS, Pan WA, Trejo J. GPCRs in cancer: protease-activated receptors, endocytic adaptors and signaling. *Int J Mol Sci.* 2018;19:1886. doi:10.3390/ijms19071886.
- Xiao T, Zhang Q, Zong S, Zhong WL, Qin Y, Bi Z, et al. Protease-activated receptor-1 promotes epithelial-endothelial transition through Twist1 in hepatocellular carcinoma. *J Exp Clin Cancer Res.* 2018;37:185. doi:10.1186/s13046-018-0858-4.
- Sebert M, Sola-Tapias N, Mas E, Barreau F, Ferrand A. Protease-activated receptors in the intestine: focus on inflammation and cancer. *Front Endocrinol.* 2019;10:717. doi:10.3389/fendo.2019.00717.
- Shavit-Stein E, Sheinberg E, Golderman V, Sharabi S, Wohl A, Gofrit SG, et al. A novel compound targeting protease receptor 1 activators for the treatment of glioblastoma. *Front Neurol.* 2018;9:1087. doi:10.3389/fneur.2018.01087.
- Galmiche A, Rak J, Roumenina LT, Saidak Z. Coagulome and the tumor microenvironment: an actionable interplay. *Trends Cancer.* 2022;8:369–83. doi:10.1016/j.trecan.2021.12.008.
- Oshi M, Sarkar J, Tokumaru Y, Yan L, Kosaka T, Akiyama H, et al. Higher intra-tumoral expression of pro-coagulation genes is a predictor of angiogenesis, epithelial mesenchymal transition and worse patient survival in gastric cancer. *Am J Cancer Res.* 2022;12:4001–14.
- Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, Drill E, et al. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell.* 2018;173:291–304.e6. doi:10.1016/j.cell.2018.03.022.
- Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics datasets—update. *Nucleic Acids Res.* 2013;41:D991–5. doi:10.1093/nar/gks1193.
- Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res.* 2017;77:e108–10. doi:10.1158/0008-5472.CAN-17-0307.
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209–49. doi:10.3322/caac.21660.
- Yang WJ, Zhao HP, Yu Y, Wang JH, Guo L, Liu JY, et al. Updates on global epidemiology, risk and prognostic factors of gastric cancer. *World J Gastroenterol.* 2023;29:2452–68. doi:10.3748/wjg.v29.i16.2452.
- Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. *Gastroenterology.* 2017;153:420–9. doi:10.1053/j.gastro.2017.04.022.
- Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. *Lancet.* 2016;388:2654–64. doi:10.1016/s0140-6736(16)30354-3.
- GASTRIC Group; Paoletti X, et al. Benefit of adjuvant chemotherapy for resectable gastric cancer. *JAMA.* 2010;303:—. doi:10.1001/jama.2010.534.
- Claassen YHM, van Amelsfoort RM, Hartgrink HH, Dikken JL, de Steur WO, van Sandick JW, et al. Effect of hospital volume with respect to performing gastric cancer resection on recurrence and survival. *Ann Surg.* 2019;270:1096–102. doi:10.1097/sla.0000000000002940.
- Chittawadagi B, Nayak SK, Ramakrishnan P, Kumar S, Cumar B, Natarajan R, et al. Laparoscopic D2 gastrectomy in advanced gastric cancer: postoperative outcomes and long-term survival analysis. *Asian J Endosc Surg.* 2021;14:707–16. doi:10.1111/ases.12926.
- Ito Y, Miyashiro I, Ishikawa T, Akazawa K, Fukui K, Katai H, et al. Determinant factors on differences in survival for gastric cancer

- between the United States and Japan using nationwide databases. *J Epidemiol.* 2021;31:241–8. doi:10.2188/jea.JE20190351.
29. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature.* 2000;407:258–64. doi:10.1038/35025229.
 30. Otsuki T, Fujimoto D, Hirano Y, Goi T, Yamaguchi A. Thrombin conducts epithelial–mesenchymal transition via protease-activated receptor-1 in human gastric cancer. *Int J Oncol.* 2014;45:2287–94. doi:10.3892/ijo.2014.2651.
 31. Yu M, Chen Y, Wang Z, Ding X. pHLIP(Var7)-P1AP suppresses tumor cell proliferation in MDA-MB-231 triple-negative breast cancer by targeting protease activated receptor 1. *Breast Cancer Res Treat.* 2020;180:379–84. doi:10.1007/s10549-020-05560-2.
 32. Arakaki AKS, Pan WA, Trejo J. GPCRs in cancer: protease-activated receptors, endocytic adaptors and signaling. *Int J Mol Sci.* 2018;19. doi:10.3390/ijms19071886.
 33. Xiao T, Zhang Q, Zong S, Zhong WL, Qin Y, Bi Z, et al. Protease-activated receptor-1 promotes epithelial-endothelial transition through Twist1 in hepatocellular carcinoma. *J Exp Clin Cancer Res.* 2018;37:185. doi:10.1186/s13046-018-0858-4.
 34. Sebert M, Sola-Tapias N, Mas E, Barreau F, Ferrand A. Protease-activated receptors in the intestine: focus on inflammation and cancer. *Front Endocrinol (Lausanne).* 2019;10:717. doi:10.3389/fendo.2019.00717.
 35. Shavit-Stein E, Sheinberg E, Golderman V, Sharabi S, Wohl A, Gofrit SG, et al. A novel compound targeting protease receptor 1 activators for the treatment of glioblastoma. *Front Neurol.* 2018;9:1087. doi:10.3389/fneur.2018.01087.
 36. Galmiche A, Rak J, Roumenina LT, Saidak Z. Coagulome and the tumor microenvironment: an actionable interplay. *Trends Cancer.* 2022;8:369–83. doi:10.1016/j.trecan.2021.12.008.
 37. Oshi M, Sarkar J, Tokumaru Y. Higher intra-tumoral expression of pro-coagulation genes is a predictor of angiogenesis, epithelial mesenchymal transition and worse patient survival in gastric cancer. *Am J Cancer Res.* 2022;12:4001–14.
 38. Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, Drill E, et al. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell.* 2018;173:291–304.e6. doi:10.1016/j.cell.2018.03.022.
 39. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics datasets—update. *Nucleic Acids Res.* 2013;41:D991–5. doi:10.1093/nar/gks1193.
 40. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res.* 2017;77:e108–10. doi:10.1158/0008-5472.CAN-17-0307.
 41. He Q, Yang J, Jin Y. Immune infiltration and clinical significance analyses of the coagulation-related genes in hepatocellular carcinoma. *Brief Bioinform.* 2022;23:4–11. doi:10.1093/bib/bbac291.
 42. Tsai MJ, Chang WA, Jian SF, Chang KF, Sheu CC, Kuo PL. Possible mechanisms mediating apoptosis of bronchial epithelial cells in chronic obstructive pulmonary disease – a next-generation sequencing approach. *Pathol Res Pract.* 2018;214:1489–96. doi:10.1016/j.prp.2018.08.002.
 43. Zhang W, Liu S, Zhan H, Yan Z, Zhang G. Transcriptome sequencing identifies key pathways and genes involved in gastric adenocarcinoma. *Mol Med Rep.* 2018. doi:10.3892/mmr.2018.9370.
 44. Wagner EF, Nebreda ÁR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer.* 2009;9:537–49. doi:10.1038/nrc2694.
 45. Sutherland TE, Dyer DP, Allen JE. The extracellular matrix and the immune system: a mutually dependent relationship. *Science.* 2023;379:11–5. doi:10.1126/science.abp8964.
 46. Cui SX, Yu XF, Qu XJ. Roles and signaling pathways of des-γ-carboxyprothrombin in the progression of hepatocellular carcinoma. *Cancer Invest.* 2016;34:459–64. doi:10.1080/07357907.2016.1227445.
 47. Bernegger S, Jarzab M, Wessler S, Posselt G. Proteolytic landscapes in gastric pathology and cancerogenesis. *Int J Mol Sci.* 2022;23. doi:10.3390/ijms23052419.
 48. Adams GN, Rosenfeldt L, Frederick M, Miller W, Waltz D, Kombrinck K, et al. Colon cancer growth and dissemination relies upon thrombin, stromal PAR-1, and fibrinogen. *Cancer Res.* 2015;75:4235–43. doi:10.1158/0008-5472.CAN-15-0964.

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YW: data collection, assembly of data, writing and final approval.

MW: data collection, assembly of data, analysis, interpretation, writing and final approval.

YZ: Data analysis, interpretation, writing and final approval.

FZ: Administrative support, provision of study materials or patients, writing and final approval.

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