Function of fibroblast growth factor 2 in gastric cancer occurrence and prognosis

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Abstract. The present study aimed to explore the role of fibroblast growth factor 2 (FGF2) in the development and prognosis of gastric cancer (GC). The relationship between FGF2 mRNA expression levels and the clinical characteristics of GC was investigated using microarray data from four GC cohorts involving 726 patients obtained from the Gene Expression Omnibus. The results of the present study indicated that FGF2 expression levels were an independent factor affecting the prognosis of GC. The primary functions of FGF2 were related to cell adhesion and angiogenesis, and patients with high levels of FGF2 expression had poorer TNM staging and prognosis; these differences were statistically significant. In terms of immune infiltration, a higher extent of M2 macrophage intrusion was observed in patients with higher levels of FGF2. However, the degree of infiltration by dendritic and $CD4^+$ T cells was lower, and this difference was statistically significant. Multivariate Cox proportional hazards model analysis revealed that age, TNM staging and FGF2 expression levels were independent prognostic factors for GC. In summary, FGF2 expression was demonstrated to be an independent prognostic factor in GC, and higher levels of FGF2 may promote the progression of this malignancy.

Introduction

Gastric cancer (GC) is a common malignant tumor with the fourth-highest occurrence among different types of cancer, and it is the third leading cause of death worldwide (1,2). Even following diagnosis at an early stage, where endoscopic mucosal resection or endoscopic submucosal dissection are successfully performed, the recurrence rate and prognosis are unsatisfactory (3). Incomplete assessment or resection can lead to the local recurrence and worse prognosis. In addition, various factors, including angiogenesis and tumor cell adhesion, are associated with the development of GC (4,5). Therefore, it is important to identify novel diagnostic and prognostic markers for guiding or assessing the treatment of GC. In addition, transcriptome analysis using microarray and RNA sequencing has been previously shown to be an effective method to identify biomarkers (6,7).

As a member of the fibroblast growth factor (FGF) family, FGF2 has important roles in angiogenesis, the regulation of extracellular matrix, cell differentiation and inflammatory responses, thereby contributing to the development, progression and pathogenesis of tumors (8-10). FGF2 has been reported to mediate cell migration and invasion in breast cancer, pancreatic cancer, astrocytes and gliomas (11-14). however, whether FGF2 has a role in the occurrence and development of GC remains unclear. In the present study, microarray data from patients with GC from Gene Expression Omnibus (GEO) databases were analyzed in order to investigate the function of FGF2 in GC.

Materials and methods

Microarray data collection. To identify GC data sets with relevant clinical information, systematic searches of GEO datasets (https://www.ncbi.nlm.nih.gov/geo/) were conducted. The inclusion criteria for the datasets were as follows: i) Sample size >50; ii) the studies presented relevant clinical information; and iii) datasets were generated using Affymetrix Human Genome 133 plus 2.0 Gene Chips (Affymetrix; Thermo Fisher Scientific, Inc.). In total, four data sets, GSE66229 (Cristescu *et al*, 2015) (n=400) (15), GSE15459 (Ooi *et al*, 2009) (n=200) (16), GSE57303 (Qian *et al*, 2014) (n=70) (17) and GSE34942 (Lei *et al*, 2012) (n=56) (18), were selected for further analysis. The batch function of SVA package (3.32.1 version) (https://bioconductor.org/packages/release/bioc/html/sva.html) was used to consolidate the 4 data sets. Data normalization was performed by R software (3.6.0 version; https://cran.r-project.org/) and gene expression levels were computed as mean values of all annotated probe sets (19).

Analysis of correlation between FGF2 expression and clinical characteristics. The mRNA expression levels of FGF2 between tumor (n=626) and normal tissues (n=100) were compared. Both the median and the receiver operating

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characteristic (ROC) curve can be used as the grouping criteria. Due to the difference in the sample size between the groups, the median expression of FGF2 was selected to replace the ROC curve as the grouping standard. According to the median expression of FGF2, 612 patients, whose clinical information was available, were equally divided into two groups: An FGF2-high expression group (FGF2-H) and an FGF2-low expression group (FGF2-L). Differentially expressed genes [P<0.05, and |log fold change (FC)|≥1)] were identified between the two groups and analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) using DAVID 6.8 (https://david.ncifcrf.gov/) (20). Clinical characteristics, including patient age, gender, tumor stage, Lauren classification (21), the extent of immune infiltration and overall survival (OS), were collected in order to identify correlations regarding FGF2 expression and clinical characteristics. The CIBERSORT (https://cibersort.stanford.edu/) method and the LM22 gene signature were used for immune infiltration analysis (22). All results were considered statistically significant at a threshold of P<0.05.

Construction of a clinical prognosis model. A Cox-prognosis model was constructed using R software (3.6.0 version). R packages including survival (version 2.44-1.1; https://github.com/therneau/survival), survminer (version 0.4.5; http://www.sthda.com/english/rpkgs/survminer/), survivalROC (contained in survcomp package; version 1.34.0; https://git.bioconductor.org/packages/survcomp) and survcomp (version 1.34.0; https://git.bioconductor.org/packages/survcomp) were used for the model and the calculation of C-index. In total, 612 patients with clinical information were randomly divided into two groups: A training group (n=306) and a validation group (n=306). The risk score for each patient was calculated based on the independent factors identified by the multivariate Cox model. The prognosis for each patient was evaluated according to risk scores. The sensitivity and specificity of the model were described using a time-dependent ROC curve (23).

Statistical analysis. Statistical analysis was performed using R software and SPSS version 23.0 (IBM Crop.). Graphical representations were generated using GraphPad Prism 7 (GraphPad Software, Inc.). χ^2 and Wilcoxon rank-sum tests were used for categorical and continuous variables, respectively. Multivariate Cox proportional-hazards analysis was used for identifying independent prognostic factors.

Results

Differential gene expression. In total, four GEO datasets were extracted and normalized to analyze the expression patterns of FGF2 in GC. Compared with normal tissues, FGF2 mRNA expression levels were lower in GC (P<0.0001; Fig. 1). A total of 536 differentially expressed genes in the FGF2-H and FGF2-L cohorts were identified by R software and the results were analyzed using GO and KEGG (Fig. 2 and Table SI). GO analysis results revealed that the differentially expressed genes were predominantly involved in the regulation of 'extracellular matrix organization', 'cell adhesion' and 'angiogenesis' (Fig. 3A). KEGG analysis results revealed that the genes identified were predominantly involved in 'focal adhesion', 'ECM-receptor interaction' and the 'cGMP-PKG signaling pathway' (Fig. 3B). The biological functions and signaling pathways associated with these genes were closely related to the development of GC.

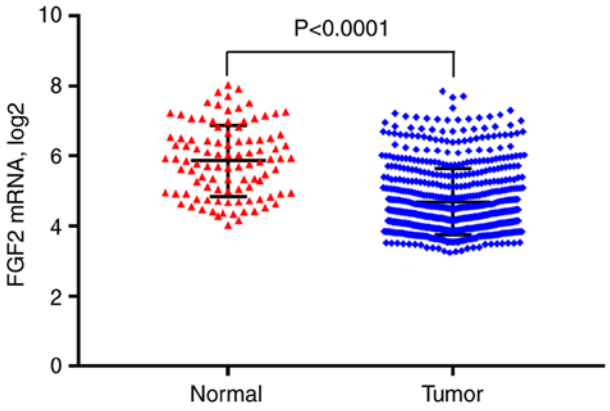

Figure 1. FGF2 mRNA expression levels in normal and gastric cancer tissues. FGF2, fibroblast growth factor 2.

Association of FGF2 expression with clinical characteristics. In total, 612 patients with clinical data were selected, and an association analysis of the FGF2 expression levels and clinical characteristics was performed. The patients were divided to the FGF2-H and FGF2-L groups, according to the median FGF2 expression. As listed in Table I, the patient age in the FGF2-L group was higher compared with that in the FGF2-H group. A statistical significance was found between stages II and IV (P<0.001; Table I), therefore, for further comparison, stages I and II were classified as early-stage and stages III and IV as advanced. The FGF2-H group had more patients with advanced-stage tumors compared with the FGF2-L group (P<0.001; Table I). Classifying the patient samples according to Lauren's criteria, a difference was observed in diffuse and intestinal types, but no difference was observed for the mixed types. In total, 366 patients were selected with detailed TNM staging in the four datasets for further analysis. There was a significant difference between the two FGF2-expressing groups in the T2 and T3 stages. Compared with FGF2-L, the number of patients with advanced-stage GC (T3 and T4) in the FGF2-H group was higher compared with the FGF2-L group (P<0.001; Table I). No significant difference was observed when comparing the N and M scores of the two FGF2-expressing groups (Table I).

Comparison of immune cell type fractions. Patients with P>0.05 were removed following CIBERSORT calculations (n=584; FGF2-H=301, FGF2-L=283), and non-parametric testing was used for comparison of the immune cell fractions (Figs. 4 and 5). The results revealed significant differences among the abundance of various immune cells, including $CD4^+$ T cells, T regulatory cells, γδ T cells, macrophages, dendritic cells and mast cells (P<0.05).

High FGF2 expression predicts poorer OS in patients with GC. Since FGF2 has a role in cell adhesion and because significant differences were identified in the abundance of different

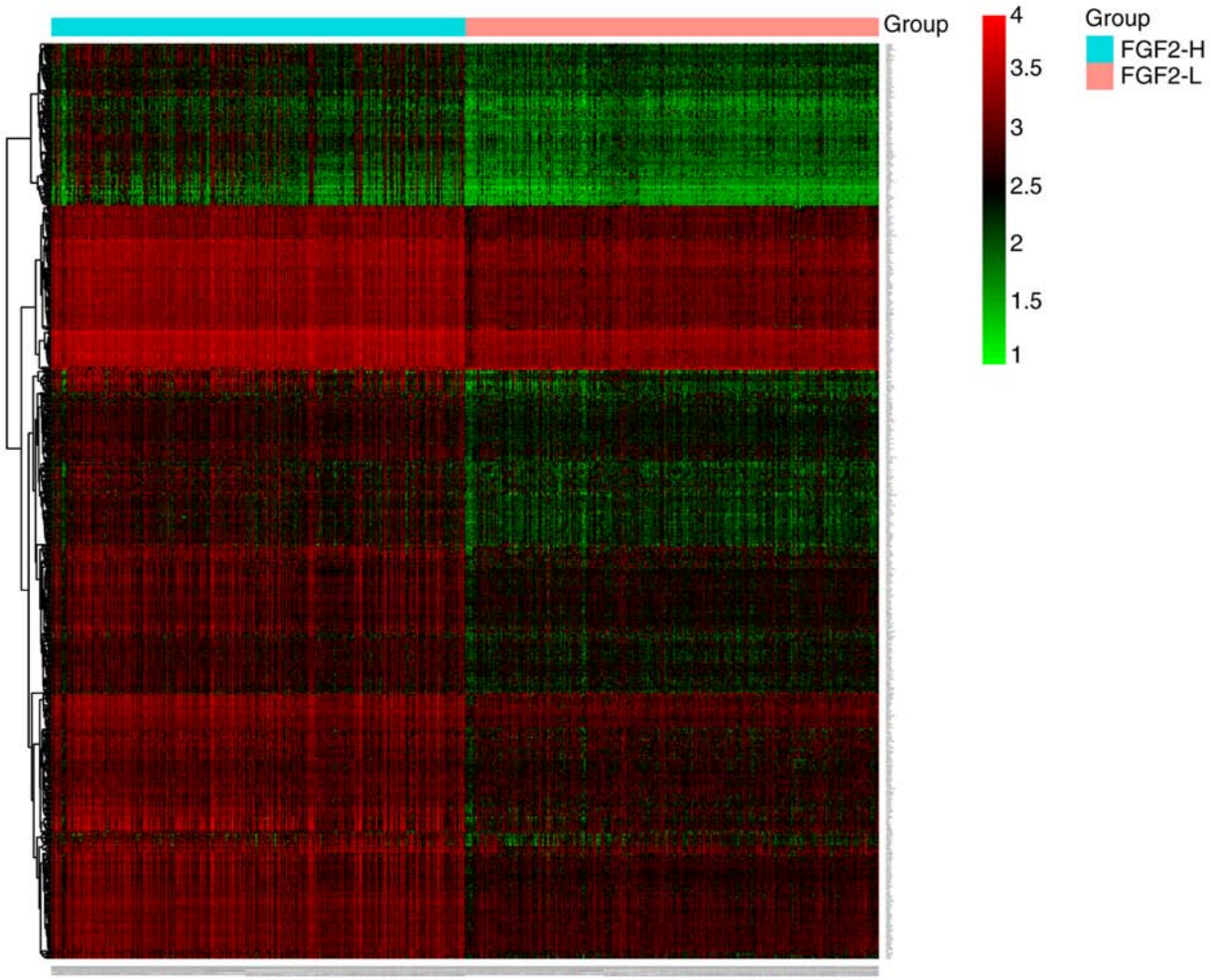

Figure 2. Heatmap of differentially expressed genes in the FGF-H and FGF-L groups. Expression level was converted by log2 method. FGF2, fibroblast growth factor 2; H, high; L, low.

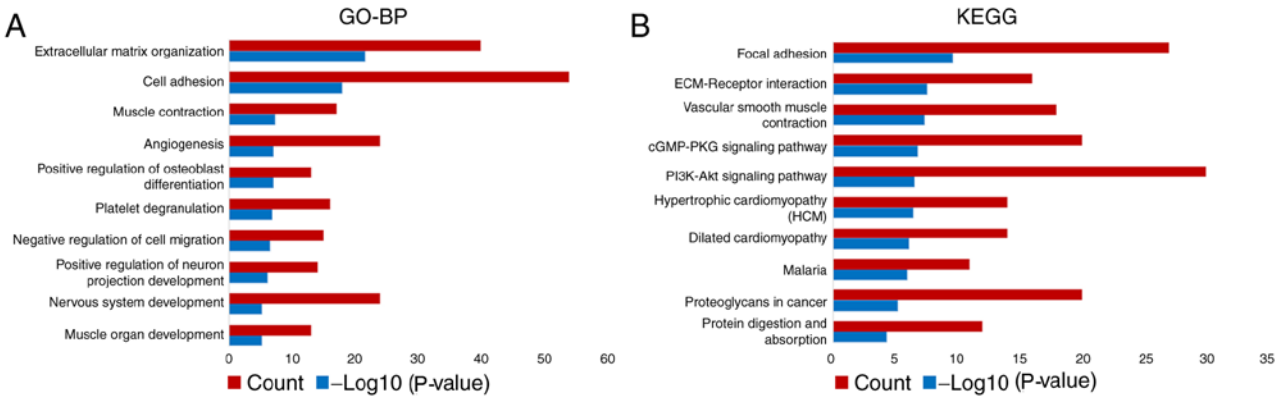

Figure 3. GO and KEGG terms enriched in the differentially expressed genes. (A) GO analysis of differentially expressed genes revealed that FGF2 was predominantly involved in 'extracellular matrix organization', 'cell adhesion' and 'angiogenesis'. (B) KEGG analysis revealed that the differentially expressed genes were predominantly involved in 'focal adhesion', 'ECM-receptor' interaction and the 'cGMP-PKG signaling pathway'. FGF2, fibroblast growth factor 2; GO, gene ontology; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

immune cell types in the tumor tissues, the survival of patients with GC may be affected by FGF2 expression levels. As shown in Fig. 6A, there was a strong association between higher FGF2 expression and shorter OS in patients with GC. Using multivariate analysis, FGF2 levels, age and tumor staging, were demonstrated to be independent prognostic indicators (Fig. 6B).

Clinical prognosis model with independent values. The risk score for each patient in the training group (n=306) and the validation group (n=306) was calculated based on the three independent factors, FGF2 levels, age and tumor staging, as identified by the multivariate Cox model (Fig. 6B). The survival analysis revealed that patients with low-risk scores

Table I. Clinical characteristics and association with FGF2 expression.

	FGF2-H group	FGF2-L group	P-value
Age (n=612)			0.0043
≥60	191	224	
<60	115	82	
Sex (n=612)			0.1227
Female	111	93	
Male	195	213	
TNM stage (n=612)			
I	25	50	0.0021
II	55	90	0.0009
III	125	102	0.4706
IV	101	64	0.0008
I+II/III+IV	80/226	140/166	<0.0001
Lauren (n=612)			
Diffuse	165	90	<0.0001
Intestinal	114	189	<0.0001
Mixed	27	27	1.0000
T (n=366)			
T1	0	0	1.0000
T2	64	130	<0.0001
T3	103	39	<0.0001
T4	16	14	0.7031
T1+2/T3+4	64/119	130/53	<0.0001
N (n=366)			
N0	27	24	0.6507
N1	70	86	0.0908
N2	55	50	0.5634
N3	31	23	0.2384
N0+1/N2+3	97/86	110/73	0.1704
M (n=366)			0.1497
M0	162	170	
M1	21	13	

FGF2, fibroblast growth factor 2; H, high; L, low.

had longer survival times in both the training and validation groups (P<0.001; Fig. 7A). ROC curves for the prognosis model were plotted, and the area under the curve was 78.2% in the training group and 82.8% in the validation group (Fig. 7B). The C-index was 0.725 in the training group [Hazard ratio (HR), 0.688-0.761; P<0.001] and 0.733 in the validation group (HR, 0.695-0.771; P<0.001). The results of the risk score and status plots (Fig. 7C and D) were consistent, and the accuracy and feasibility of the model were verified.

Discussion

GC is a common malignant tumor, with complex factors leading to high mortality and low 5-year survival rates. With technological developments, chromatin immunoprecipitation and sequencing technologies have improved, and increasing numbers of studies are now being conducted to understand in-depth the molecular mechanisms involved in GC (24).

FGF2 is a member of the FGF family and has important roles in various biological processes, such as angiogenesis, the regulation of the extracellular matrix, epithelial cell differentiation and inflammatory responses (8-10). In normal tissues, FGF2 promotes endothelial cell migration, smooth muscle proliferation and induces hematopoiesis (25,26). however, in tumor tissues, the roles of FGF2 in promoting angiogenesis and regulating cell adhesion may contribute to tumor progression (8-10). Previous studies have demonstrated that FGF2 activates cell proliferation and invasion through the PI3K/AKT, ERK or mitogen-activated protein kinase signaling pathways in esophageal, ovarian and breast cancers (27-29). Therefore, FGF2 serves important roles in the occurrence and development of tumors, and is closely associated with the activation of multiple signaling pathways. However, the role of FGF2 in the occurrence and prognosis of GC is not clear.

In the present study, the expression of FGF2 was higher in normal tissues compared with tumor tissues. Although the expression of FGF2 was found to be higher in normal tissues, the expression of FGF2 was identified to be associated with worse TNM staging using GEPIA; suggesting the need of further research to investigate the role of FGF2 in tumor. TNM stage is a significant prognostic factor in tumor progression (30,31). A comparison between FGF2-H and FGF2-L groups revealed more patients with advanced TNM staging, especially T stage, in the FGF2-H group. Previous studies have shown that FGF2, by promoting angiogenesis, allows gastric tumor cells to receive abundant nutrient supplies, thereby inducing a greater degree of tumor cell infiltration (8-10). In terms of N and M stages, though no significant differences were observed between the two groups in the present study, it was evident that the number of patients staged as N2 and N3 were higher in the FGF2-H group compared with the FGF2-L group. Similar results for the M stage of patients with GC were observed, indicating that FGF2 may affect to some extent the local and distant metastasis in GC. Further analysis involving a larger number of patients is required to confirm these observations.

In the present study, tumor immune infiltration was also evaluated, another important characteristic of GC. The degree of immune cell infiltration has different effects on tumor prognosis and has been studied previously (32-36). Preliminary analysis of the immune infiltration was conducted, no further analysis was performed as the main functional enrichment of genes in the FGF2-H and FGF2-L groups did not identify immune associated genes.

In the present study, there were significant differences in the proportion of $CD4^+$ T-cells, M2 macrophages, mast cells and dendritic cells between the two groups. Specifically, the FGF2-H group had significantly higher proportions of resting $CD4^+$ memory cells, γδ T cells, M2 macrophages and natural killer resting cells compared with the FGF2-L group, while the proportion of plasma cells, activated $CD4^+$ memory cells, M0 macrophages and activated dendritic cells was lower than in the FGF2-L group. Previous studies have reported a negative correlation between the degree of infiltration of dendritic cells, tumor progression and metastasis (37,38). Therefore,

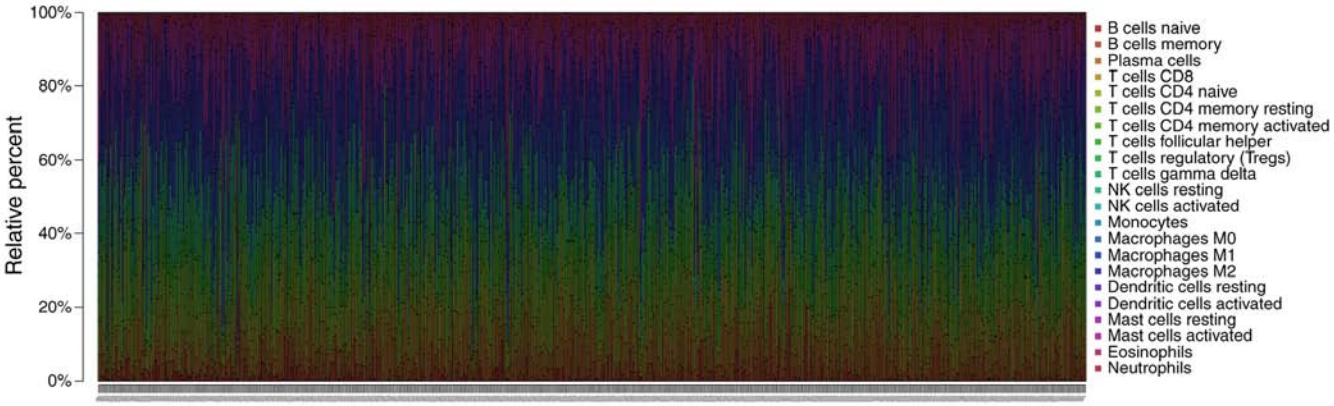

Figure 4. Comparison of the immune cell fractions in each patient.

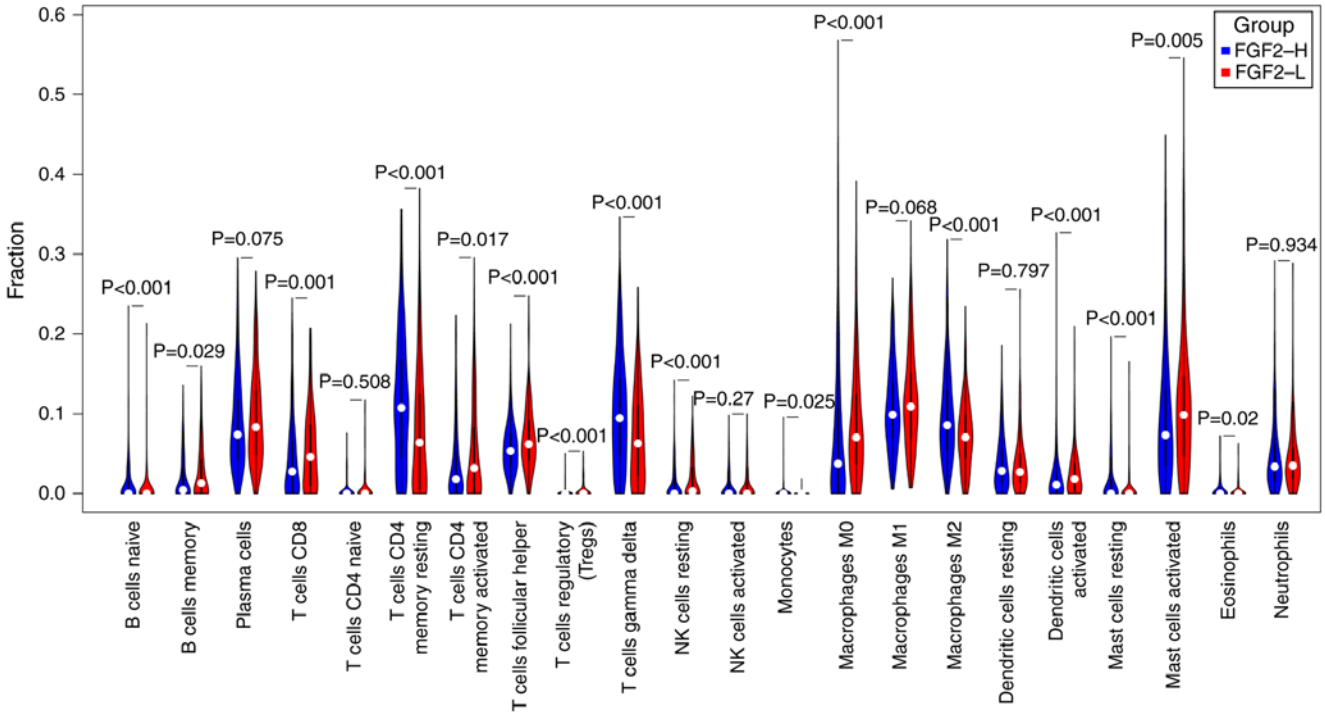

Figure 5. A non-parametric test of immune cell fractions between the FGF2-H and FGF2-L groups. FGF2, fibroblast growth factor 2; H, high; L, low.

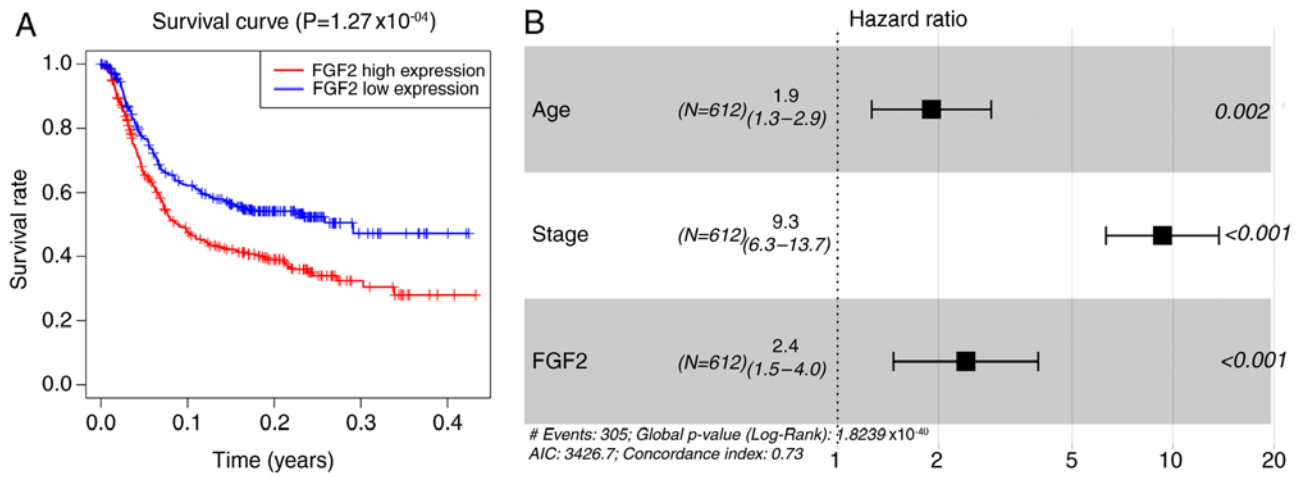

Figure 6. Survival analysis. (A) Kaplan-Meier survival plots of the FGF2-H and FGF2-L groups. (B) Forest plots showing that age, TNM staging and FGF2 expression were independent factors of GC prognosis. GC, gastric cancer; FGF2, fibroblast growth factor 2.

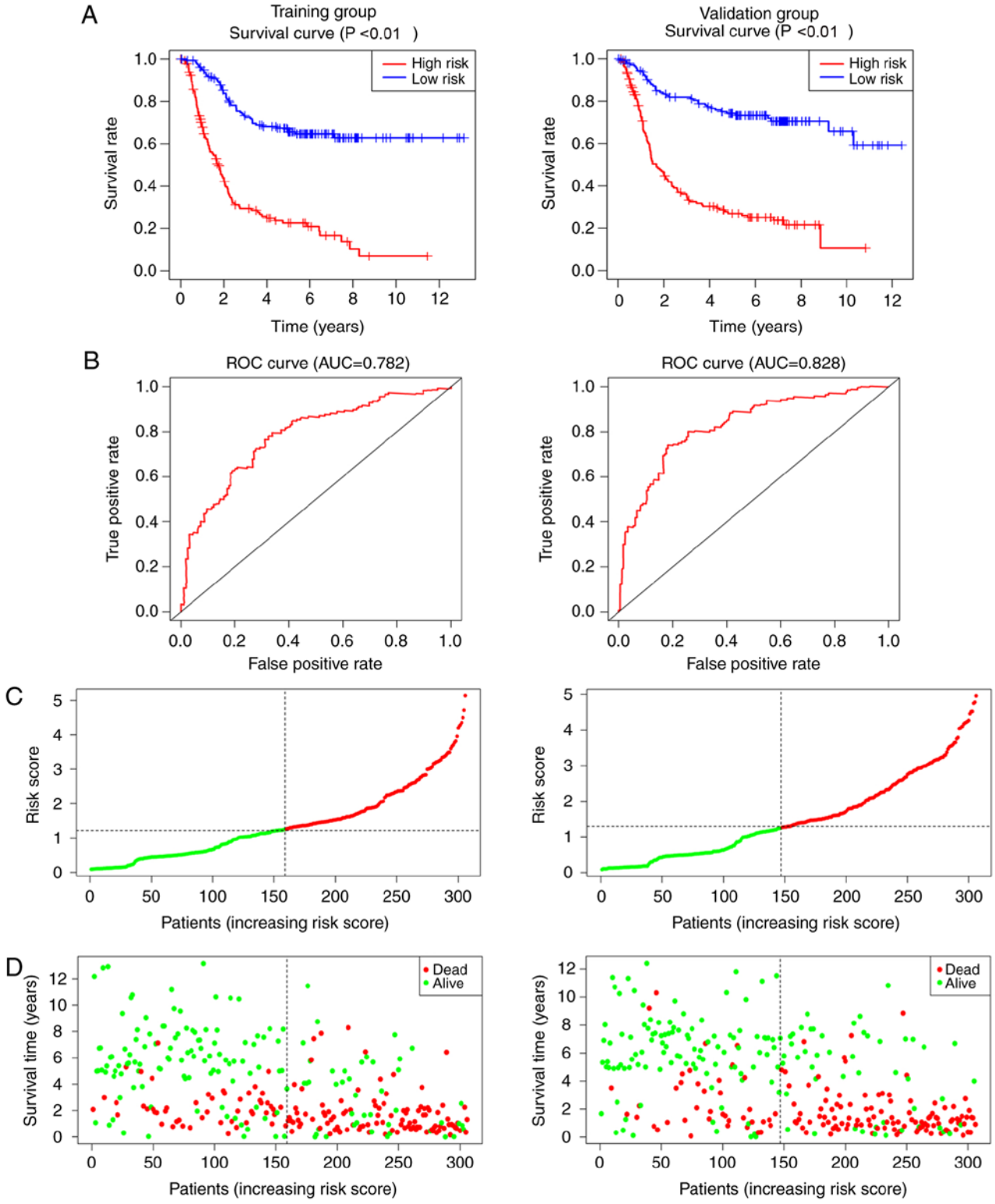

Figure 7. Clinical prognosis model for gastric cancer. (A) Kaplan-Meier survival plots, (B) ROC curves, and (C) risk scores for the training and validation groups. (D) Status plot for the training and validation groups. ROC, receiver operating characteristic.

the higher the number of dendritic cells, the more positive the prognosis for the patient. M2 macrophages are hypothesized to promote tumor growth, infiltration and metastasis, and their degree of infiltration is negatively correlated with the prognosis of patients (39,40). $CD4^+$ T cells are important for the protection against cancer (41). In the present study, patients in the FGF2-L group had more dendritic cells, $CD4^+$ memory activated T cells, fewer M2 cells, and a higher survival rate. These findings are consistent with the hypothesis that FGF2 may influence the prognosis of GC.

Using multivariate regression analysis, it was found that the survival time of patients in the FGF2-L group was significantly improved compared with the FGF2-H group. Age, TNM stage and FGF2 expression were independent factors for

GC. With the construction of a Cox risk model, the clinical prognosis of patients was determined using their risk scores. For the training and validation groups, the ROC curves were 0.78 and 0.82, respectively, indicating the high accuracy of the current model. Additionally, patients with higher risk scores had poorer survival rates.

In summary, FGF2 levels may have an important role in the development of GC and may serve as an independent factor to evaluate the prognosis of patients with GC. Further studies, increasing the sample size and using verified clinical data, are essential to understand the molecular mechanisms involved in the prognosis of GC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the GEO database.

Authors' contributions

YL and LL analyzed the data and drafted the manuscript. XG and JW evaluated and interpreted the data critically. XG, JW and HW evaluated and interpreted the data critically. LL revised and approved the final version of the manuscript. All authors critically revised the manuscript, and read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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