Research article

Identification of cancer-associated fibroblast-related Ectodysplasin-A as a novel indicator for prognosis and immune response in gastric cancer

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A R T I C L E I N F O

A B S T R A C T

Studies have indicated cancer-associated fibroblasts (CAFs) could have a significant impact in gastric cancer (GC) progression and chemotherapy resistance. However, the gene related to cancer fibroblasts that can be used as biomarkers to judge the occurrence of gastric cancer has not been fully explored. Based on two Gene Expression Omnibus (GEO) datasets, we focus on differentially expressed genes which may act as CAFs markers related to GC. Through COX regression, LASSO regression and Kaplan-Meier survival analysis, we discovered three upregulated genes (GLT8D2, GNAS and EDA) associated with poor GC patients' survival. By single-cell analysis and nomogram, we found that EDA may affect fibroblast production and disease prognosis in GC patients. EDA expression showed a positive correlation with 5-Fluorouracil IC50 values. Immunohistochemistry (IHC) and real time PCR indicated elevated EDA levels in GC tissues and cells. Enrichment analysis revealed that EDA was closely linked to immune system regulation. IHC and single-cell analysis indicated that EDA gene was associated with cancer fibroblasts marker FGF12 and influence cell interferon-gamma response, which may play a role in regulating immune-related characteristics. In summary, we concluded that EDA may be used as a new therapeutic CAFs marker for GC.

1. Introduction

Gastric cancer (GC) is among the most prevalent malignant tumors globally. It is often difficult to diagnose in the early stage, more than half of the patients with early gastric cancer are asymptomatic [1]. Early gastric cancer is usually treated by surgical resection, while patients with advanced gastric cancer are suggested continuous chemotherapy treatment with fluoropyrimidine and platinum double drug or other target drugs [2]. Apart from surgical removal of tumor tissue and clearing proliferating lymph nodes, it is more important to inhibit the emergence and subsequent growth of micrometastases. One of the key drivers behind the development of neoadjuvant chemotherapy for gastric cancer is precision treatment, the pursuit of personalized chemotherapy targets [3]. The first-line chemotherapy for GC is mainly treated with platinum and 5-fluoropyrimidine, and the triple chemotherapy means

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combination of docetaxel, 5-fluorouracil, and cisplatin [1]. Even though chemotherapy can prolong survival, the average patient has a median survival of 12–15 months, new therapies will have to be introduced more effectively to improving survival. For immunotherapy, the improvement of clinical outcomes for those with gastric cancers have been found to be associated with indicators like microsatellite instability (MSI), programmed cell death 1 ligand 1(PD-L1), or tumor-mutant burden (TMB) [4].

The Ectodysplasin A (EDA) gene, part of the tumor necrosis factor (TNF) ligand family, was initially reported to trigger the formation of various ectodermal derivatives during normal prenatal development. Recent research has shown that EDA gene and its receptors can affect tumor growth, migration, differentiation and apoptosis, which in turn might regulate tumorigenesis and response for cancer therapy [5]. The two splicing variants including EDA-A1 and EDA-A2 (which has 2 fewer amino acids compared to EDA-A1), produce their biological activity by interacting with different receptor. Similar to other TNF family receptors, EDA-A1 and EDA receptor (EDAR) are trimerized after being recruited by special protein linker EDAR-associated death domain (EDARADD) linker proteins and triggers the downstream NF-κB signaling pathway. EDA-A2 exerts its biological activity through binding to distinct receptor named EDA2R, which is also referred to the X-linked ectodermal dysplasia receptor (XEDAR) [6–8].

Cancer-associated fibroblasts (CAFs) are the main components of the tumor stroma, potentially critical in GC progression and chemotherapeutic resistance. Through immunomodulation, CAFs have a distinct effect on the tumor microenvironment (TME). They can directly influence and coordinate the involvement of immune cells via secreted cytokines and surface protein or the deposition substrates and transformations of various extracellular matrix (ECM) [9,10]. The precise mechanism of function and effects for CAFs are largely unstudied regarding its differences from the normal fibroblasts (NFs). In general, CAFs contribute to tumor initiation and progression by secreting various ECM proteins and regulators. Co-injection of CAFs and tumor cells has been associated with cancer progression in human tumor. In contrast to NFs, CAFs have been demonstrated to induce cancer proliferation in benign non-tumoral epithelial cell lesions. This was initially observed in a mouse model of prostate cancer by co-injecting benign epithelial cells with CAFs [11]. The chemokine secretion is one of the mechanisms that accelerate tumor progression and invasiveness. In gastric cancer, CAFs secrete CXCL12, activating the CXCL12/CXCR4 signaling pathway to promote tumor growth and invasion [12]. Except for CXCL12 release, CAFs-affected TME remodeling also promotes tumor invasion and metastasis. The protein-rich matrix promotes GC cells into peripheral cells or metastasis to distant cells. Interestingly, CAFs can create gaps between the matrix and the base membrane that are mediated by the cell-cell interaction, and then, through matrix metalloproteinase (MMP) or independent MMP mechanisms, affect tumor cell metastasis [11]. Therefore, CAFs contribute to the proliferation and metastasis of GC tumors.

This study explores the expression of the up-regulated gene EDA in CAFs associated with GC. In GC patients, EDA expression significantly correlates with poor prognosis. Additionally, we investigated the relationship between EDA expression and the half maximal inhibitory concentration (IC50) of 5-fluorouracil (5-FU). Correlation analysis revealed a positive association between the EDA gene and CAF marker genes. Furthermore, gene-set enrichment analysis (GSEA) and single-cell analysis were conducted to predict EDA's function. Finally, we analyzed the correlation between EDA expression and the expression of specific immune inhibitors. These results suggested that EDA may be a new marker for the CAF and new immunotherapy target of gastric cancer.

2. Materials & methods

2.1. Data collection

Initially, we utilized the GEO platform [13], to analyze two gastric cancer datasets, namely GSE19426114 [14] and GSE11617615 [15] (Table 1). In addition, we identified differentially expressed genes (DEGs) related to CAFs in GC. We set |log₂FC|≥0.7 in GSE116176 and set |log₂FC|≥0.4 in GSE194261. Venn diagrams were employed to explore co-differentially expressed genes in CAFs related to GC, utilizing the VENNY 2.1 online web tool (https://bioinfogp.cnb.csic.es/tools/venny). ImageGP platform was applied to draw the heat map of all DEGs in two datasets.

2.2. Gene expression and survival analysis

After identifying differential genes, we applied several platforms to analyze gene expression and therapy response in patients with gastric cancer (Table 2). First, LASSO regression analysis was conducted to identify the prognostic genes among the differential genes. Then the prognostic effect of these genes was further confirmed by survival analysis of Kaplan-Meier plotter [16]. In addition, the prognostic value of EDA was analyzed by Xiantao academic tool and to calculate the risk score of overall survival (OS) and disease specific survival (DSS). In the statistical analysis of differential genes, the log-rank test was performed. Moreover, nomogram and calibration curve were applied to verify the results of prognostic analysis. Furthermore, we utilized the GEPIA2.0 platform [17] and UALCAN [18] to contrast EDA expression between tumor and normal groups.

Table 1
The characteristics of the two GEO datasets on gene expression profiling array.

GEO datasets	Platform	Sample size		DEGs	References
		CAF	Normal fibroblast		
GSE194261	GPL17077	1	1	6387 upregulated genes; 5423 downregulated genes	[14]
GSE116176	GPL21185	2	2	1576 upregulated genes;1282 downregulated genes	[15]

Table 2
Bioinformatics platforms have been used to evaluate the importance of EDA in gastric cancer.

Database	URL	Refs
GEO	https://www.ncbi.nlm.nih.gov/gds/?term	[13]
GEPIA2.0	http://gepia2.cancer-pku.cn/#index	[17]
UALCAN	https://ualcan.path.uab.edu/index.html	[18]
Kaplan-Meier plotter	https://kmplot.com/analysis/	[16]
Best	https://rookieutopia.com/app_direct/BEST/	
TISCH2	http://tisch.comp-genomics.org/home/	[19]
TISIDB	http://cis.hku.hk/TISIDB/	[20]
TIMER	https://cistrome.shinyapps.io/timer/	[21]
DISCO	https://www.immunesinglecell.org/	[22]

In order to explore the relationship between EDA and response to chemotherapy, we utilized the Kaplan-Meier plotter to examine EDA expression in patients treated with fluorouracil and its correlation with prognostic factors, including first progression (FP), overall survival (OS), and post-progression survival (PPS). In the OS survival analysis, we used EDA probes which included 206217_at, 211129_x_at and 211130_x_at. In survival analysis of PPS, we used EDA probes which included 211128_x_at, 211129_x_at and 211131_x_at. In survival analysis of PPS, we used EDA probes which included 211129_x_at and 211130_x_at. The treatment of patients was uniformly received 5-FU based adjuvant. Furthermore, the correlation between IC50 of 5-FU and EDA expression was explored through BEST database. Through drug susceptibility analysis, we selected the GSDC2 database for analyzing IC50 of 5-FU. The selected cohorts were GSE84437 [23] and TCGA_STAD, and the statistical method was used for Pearson correlation analysis. Similarly, we selected the GSDC1 database for gemcitabine analysis. The selected cohort was GSE183136 [24], and the statistical method was also set to Pearson correlation analysis.

2.3. Immunohistochemistry (IHC) assay

To investigate the relationship between EDA and CAFs, the marker genes of CAFs were set by searching Google scholar and Pubmed website for papers, and analyzed by the TIMER database [21] and Xiantao tool. To examine the expression of EDA and the CAFs marker FGF12 in gastric cancer, we conducted IHC analysis on tissue microarrays (Shanghai Outdo Biotech, HStmA180Su08) comparing GC tissues with normal tissues. After washing the slides with deparaffinization reagents and blocking the endogenous peroxidase activity, sections were then transferred in retrieval solution (Tris-EDTA pH: 9.0) for 3 min. Slides were then incubated with primary antibodies (EDA, Proteintech, 25892-1-AP; FGF12, Proteintech, 13784-1-AP) at 37 °C for 30 min. Following this, sections were washed with Tris-buffered saline and incubated with secondary antibodies at room temperature for another 30 min. Positive staining signals were visualized using DAB, and sections were counterstained with hematoxylin. The staining signals were scored to 6–12 (high) and 0–4 (low), according to the standard procedures. The correlation between the expression of EDA and FGF12 was assessed using violin plot and scatter plot. The survival curve was employed to investigate the relationship between EDA expression and patient survival probability.

2.4. Real time PCR

EDA mRNA expression in human GC cells (MGC-803 and AGS) and gastric mucosal cells (GES-1) was analyzed using real-time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen), following the manufacturer's instructions, and reverse-transcribed to cDNA using the PrimeScriptTM strand cDNA synthesis kit (6210, Takara). Real-time PCR was performed on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, United States) to quantify EDA expression. The forward primer sequence for EDA is "GTAGGCGTGTTCGCCGCAATAA", and the reverse primer sequence is "GTCCAGGAGGTCCTGCTTTCTT". Changes in mRNA levels relative to the reference gene GAPDH were calculated using the $2^{-\Delta\Delta ct}$ method with iTaq Universal SYBR Green Supermix (1725121, Bio-Rad).

2.5. Single cell analysis and immunotherapy responses

Single cell analysis annotated by cell type (major-lineage) was conducted using the Tumor Immune Single Cell Hub (TISCH) tool [19] based on the STAD_GSE134520 and STAD_GSE167297 data. The correlation analysis of EDA expression and TCGA survival across cancer types was also performed using the TISCH tool. The expression of EDA in reference cell type was identified by Deeply Integrated human Single-Cell Omics (DISCO) database [22] at the single cell resolution. To investigate the potential function of EDA, GO and GSEA enrichment analysis were applied through the BEST and TISCH platform. Using the BEST database, we selected 1000 genes for analysis, while setting the Q-value cut-off to 0.05. Similarly, we applied the TISCH database to perform Hallmark enrichment analysis of single cell data in the GSE134520 dataset. Interferon-gamma response was selected to conduct single-cell signature exploration to indicate different cell types respond conditions to interferon. The TISIDB platform [20] was utilized to investigate the correlation between EDA and immune inhibitors expression in stomach adenocarcinoma (STAD).

2.6. Statistical analysis

The *t-test* was used to compare differences between two groups, with statistical significance set at p < 0.05. The log-rank test assessed the relationship between EDA expression and survival probability in GC patients, with significance defined as log-rank p < 0.05. Pearson correlation analyzed the association between EDA expression and IC50, while Spearman correlation explored relationships between EDA expression and CAFs marker genes or immune inhibitors.

3. Results

3.1. The identification of the CAF-related gene EDA in gastric cancer

We analyzed two GC datasets retrieved from the GEO database to screen for differential expression genes in CAFs related to GC with the condition of |log$_2$FC|≥0.7 in GSE116176 and |log$_2$FC|≥0.4 in GSE194261. In GSE194261, we identified 6387 upregulated genes and 5423 down-regulated genes, while for GSE116176, 1576 upregulated genes and 1282 down-regulated genes were identified. As shown in Fig. 1A, we applied the Venn plot to explore the 182 co-upregulated genes and 151 co-downregulated genes in two GC datasets. To screen the prognostically significant genes, we used COX regression to identify the genes correlated to overall survival using TCGA-STAD, and then overlap these genes with co-regulated genes (Supplementary Table 1). As shown in Figs. 1B and 134 co-upregulated genes and 125 co-downregulated genes were found. After picking out these common differential genes, we applied LASSO regression to analyze the subset of differentially expressed genes that minimizes prediction error for a quantitative response variable as Fig. 1C and D. The heat map of genes with clinically significant after regression analysis is shown in Fig. 1E. The prognostic significance of these genes was further assessed using the Kaplan-Meier plotter. As shown in Fig. 1F and Supplementary Figs. 1A–B, we identified three upregulated genes (GLT8D2, GNAS and EDA) associated with poor GC patients' survival, while EDA showed the predominant value and set to additional analysis.
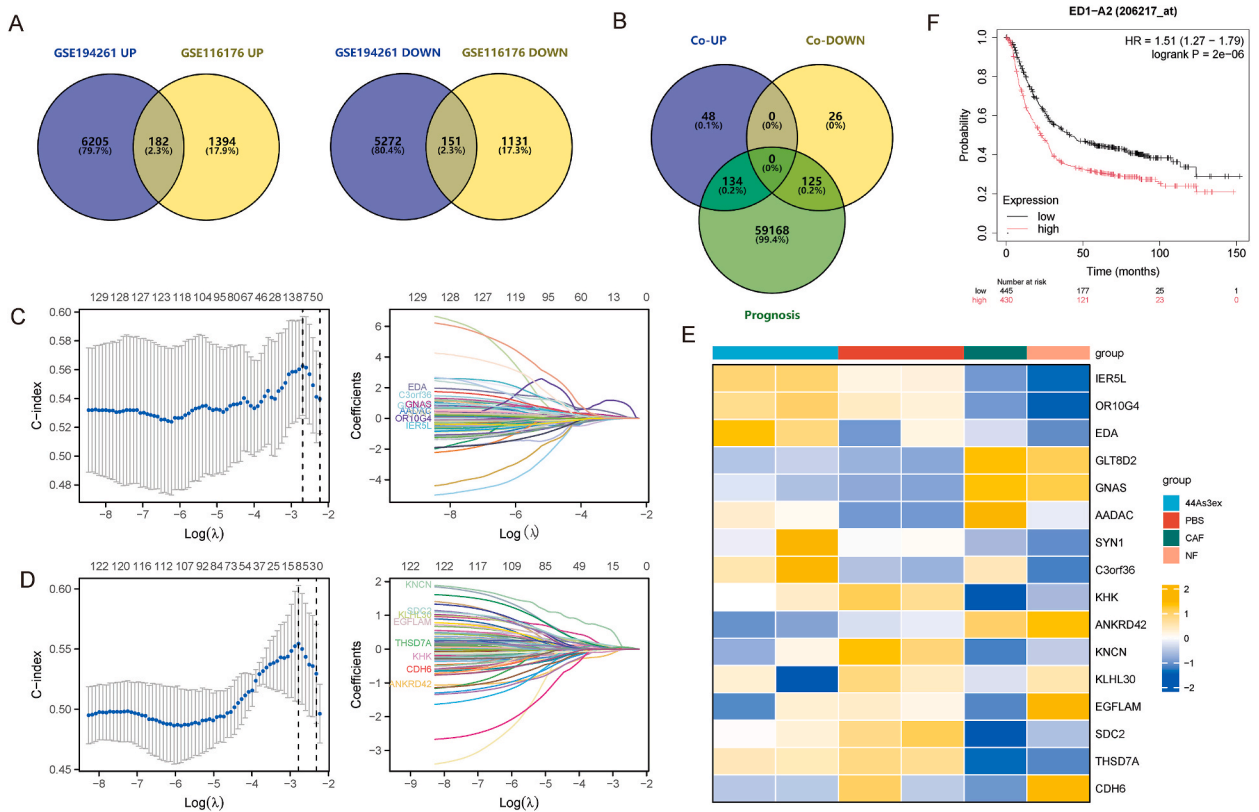
Fig. 1. Differentially expressed genes (DEGs) associated with gastric cancer fibroblasts in GSE116176 and GSE194261. (A–B) Venn diagrams were applied to analyze the co-upregulated and co-downregulated genes in GSE194261 and GSE116176. (C) Venn diagrams were applied to analyze the co-regulated genes and genes significantly associated with prognosis in TCGA_STAD. (C–D) Lasso regression analysis was further applied to identify a subset of co-DEGs related to patients' survival. (E) Heat map of clinically significant differential genes in each sample after LASSO regression analysis. (F) Analysis of the relationship between EDA expression and patient survival probability using log-rank test statistical methods with the KM database.

3.2. EDA predicts prognosis condition in GC

To further explore the predictive value of EDA, we utilized Kaplan-Meier plotter to examine its potential on OS in GC patients. As depicted in Fig. 2A, the expression of EDA predicted poor prognosis as indicated by the 211127_x_at, 211128_x_at, 211129_x_at, 211130_x_at and 211131_x_at probes. In TCGA-STAD cohort, survival analysis indicated that high expression of EDA was associated with poor prognosis in GC patients (Fig. 2B). The AUC and ROC curve were shown in Fig. 2C and D. Across variable cancer types, EDA expression significantly increased the risk in HNSC, LIHC and STAD, and decreased risk in KIRC and MESO (Fig. 2E and F). Using UALCAN [18] and GEPIA2 [17] database, EDA expression was found relatively higher in gastric cancer tissues compared to normal tissues (Supplementary Figs. 2A–B). Real-time PCR revealed that EDA expression was elevated in human GC cells (MGC-803 and AGS cells) compared to gastric mucosal cells (GES-1). (Fig. 2G). In addition, nomogram was applied to assess the potential parameters that associated with patients' survival, including EDA expression, gender, race, age, histological type and histologic grade (Supplementary Fig. 2C). The prognostic calibration curve of the nomogram observed values for 1-year, 3-year, and 5-year survival probabilities were showed in Supplementary Fig. 2D. Using DISCO database, the expression of EDA at single-cell resolution illustrated that EDA expressed in cell type such as ADAM12+ fibroblast, CFD + MGP + fibroblast and CDH19+LAMA2+ fibroblast (Fig. 2H and Supplementary Fig. 2E). Similarly, applying the TISCH tool, single-cell analysis of EDA showed its expression in fibroblast and myofibroblast (Fig. 2I). The above results revealed that EDA may be an effective marker for CAFs that leads to poor prognosis in gastric cancer patients.

3.3. EDA corelated with chemotherapy response in gastric cancer

In order to explore chemotherapy response, we utilized the BEST database to evaluate the correlation between EDA expression and chemotherapy drugs IC50. As Fig. 3A and Supplementary Fig. 3 showed, positive correlations were revealed between EDA expression
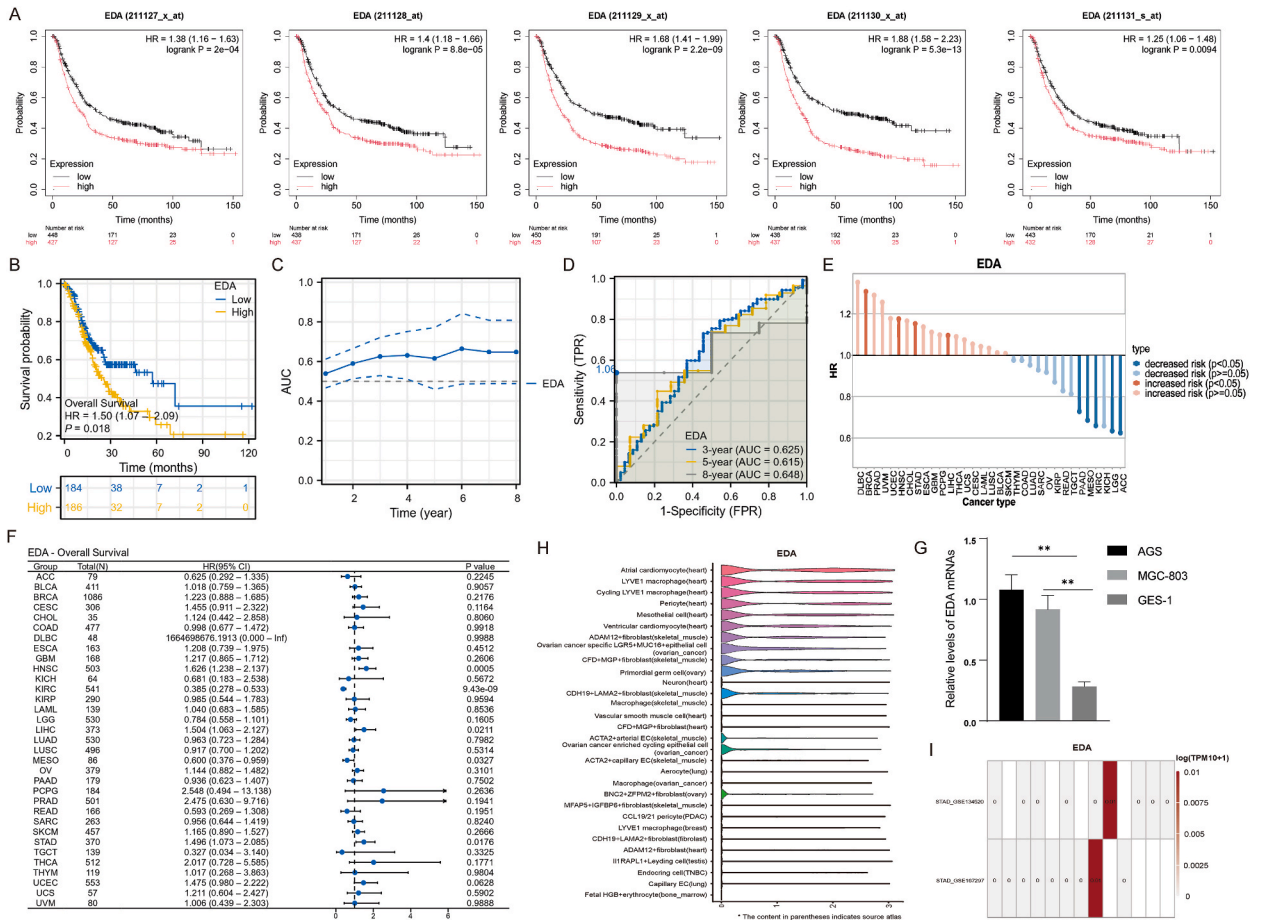

Fig. 2. EDA expressed in fibroblasts and correlated with patient prognosis. (A) Correlation analysis of the EDA gene with patients' OS using log-rank test statistical methods. (B–D) Survival, AUC, and ROC analysis of EDA gene expression with patient OS in TCGA_STAD. (E–F) Survival risk of the EDA gene in pan-cancers. (G) Real-time PCR analysis of mRNA expression in GC and normal cells. (H) The DISCO database was used to analyze the expression of EDA at single-cell resolution. (I) Applying the TISCH tool for single-cell analysis of EDA to explore its expression in GC.
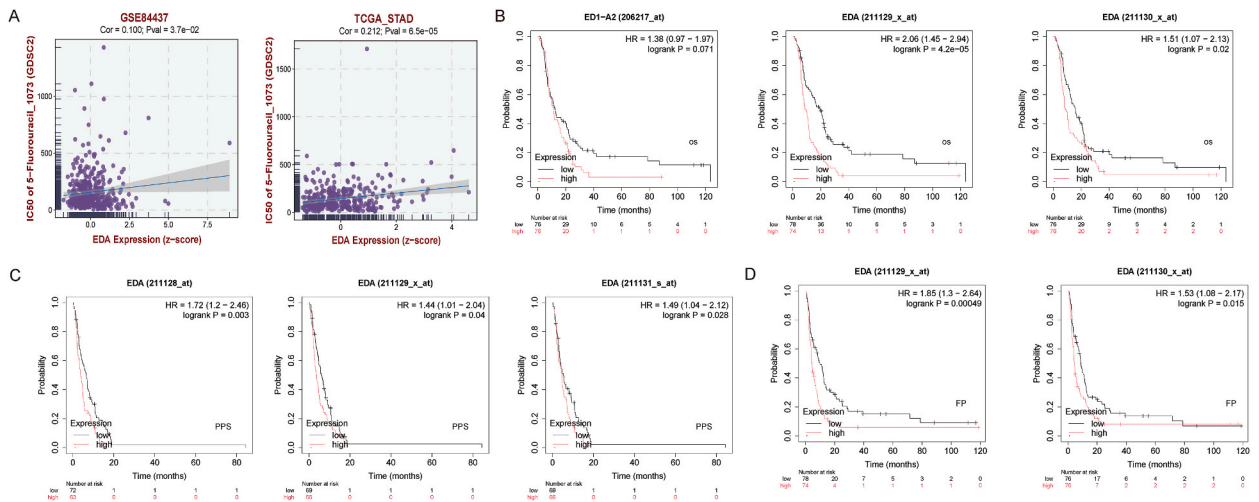
Fig. 3. Drug susceptibility and prognostic value of EDA genes. (A) The BEST database was applied to explore the correlation between EDA expression and IC50 of 5-Fluorouracil in the GSE84437 cohort and TCGA_STAD cohort of the GSDC2 database. Statistical analysis was assessed by Pearson correlation analysis. (B–D) Kaplan-Meier plotter was applied to analyze the correlation between survival probability (OS, FP, and PPS) in patients who treated with 5-FU. Statistical analysis was performed by log-rank test.

and IC50 of gemcitabine (GSE183136) and 5-FU (GSE84437 and TCGA_STAD). To further assess the prognostic values of EDA in GC, we used the Kaplan-Meier plotter to analyze its expression in patients treated with fluoride and its correlation with prognostic factors, including FP, OS and PPS. Notably, patients with higher levels of EDA gene showed shorter FP, OS, and PPS times (Fig. 3B–D). Therefore, our results suggested EDA gene has potential to serve as a biomarker for gastric cancer occurrence and as a target for pharmacological treatment, which deserves further investigation.

EDA positively associated with CAFs marker FGF12.

The CAFs are an essential component in TME. By directly or indirectly activating/deactivating other cell types, CAFs can function and promote cancer proliferation. To validate the correlation between EDA and CAFs, we utilized Xiantao tool and the TIMER database to analyze association score of EDA and CAFs markers (Fig. 4A and Supplementary Fig. 4). EDA expression was found to be positively corelated with CAFs marker genes including ADAM Metallopeptidase Domain 23 (ADAM23), Fibroblast Growth Factor 12 (FGF12), Myeloid Leukemia Factor 1 (MLF1), Plexin A1(PLXNA1), Synapse Associated Protein 1 (SYAP1), and PR/SET Domain 5 (PRDM5). Therefore, IHC analysis was employed to validate the expression of EDA and FGF12 in GC tissue compared to normal tissue (Fig. 4B). The results demonstrated significantly higher EDA and FGF12 staining in GC tissues than in normal tissues (Fig. 4C and D). Consistent with earlier findings, EDA expression showed a positive correlation with FGF12 expression in GC tissues (R2 = 0.5282, p < 0.001) (Fig. 4E). Finally, as Fig. 4F and G, similar results were found that patients with higher EDA and FGF12 expression illustrated a poorer outcome. EDA is not only significantly associated with CAFs marker genes but may also play a crucial role in patient prognosis.

3.4. Enrichment analysis of EDA in GC

To examine the biological process, cellular component, and molecular function that EDA may be involved in, we conducted the GO enrichment to predict its function of EDA using the BEST database, as shown in Fig. 5A. The results indicated that EDA is significantly involved in immune response pathways, such as immune system process and response to stimulus. The result of the GSEA enrichment analysis also showed that EDA has a strong influence on the positive regulation of multiple immune pathways, including cellular response to interferon gamma and immune response regulating cell surface receptor signaling pathway (Fig. 5B). In addition, we performed hallmark enrichment analysis through the TISCH database, and the results in Fig. 5C showed that EDA significantly associated with the interferon-gamma response pathway in fibroblasts at single cell resolution. The Stochastic neighbor Embedding (SNE) plot of EDA expression and the hallmark of response to interferon was shown in Fig. 5D and E.

3.5. Correlation between EDA with immune regulation

To investigate its effect on immune regulation, we utilized the TISIDB database to examine the relationship between EDA and immune checkpoints. The heatmap illustrating the pan-cancers analysis of relationship between immune checkpoints and EDA was shown in Fig. 6A. We found seven immune inhibitors have a negative correlation with EDA expression, and the top four immune inhibitors with the strongest correlation is CD274 (Spearman r = −0.189, p = 0.000114), IDO1 (Spearman r = −0.138, p = 0.00498), IL10RB (Spearman r = −0.139, p = 0.00466), LGALS9 (Spearman r = −0.21, p = 1.64e-5). The results are summarized in Fig. 6B–E.
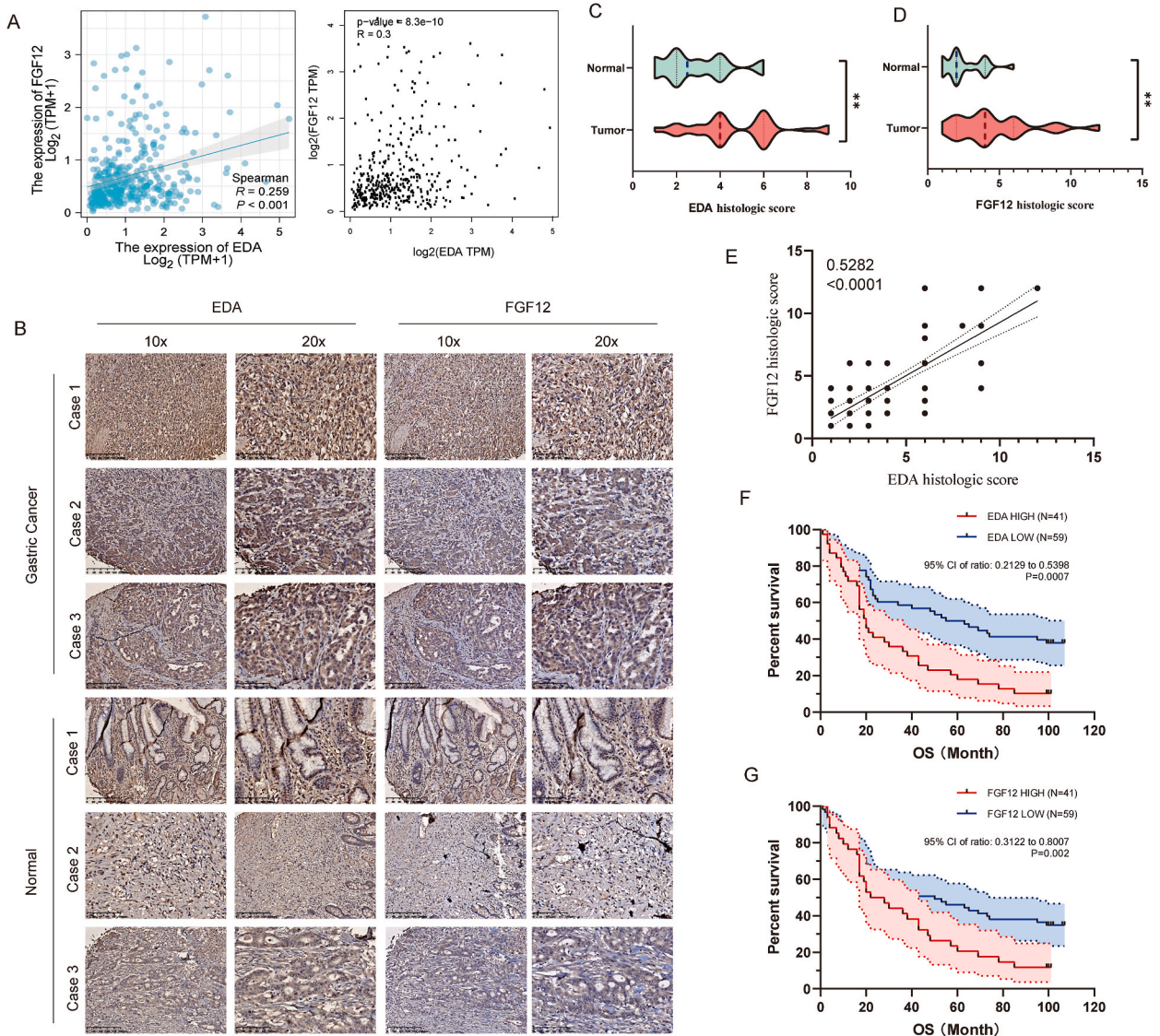
Fig. 4. Correlation analysis of EDA gene and CAFs marker genes. (A) Using the Xiantao tool and the GEPIA2 database, Spearman's rank correlation method was applied to explore the correlation between EDA and the CAFs marker gene FGF12. (B) Representative images of IHC staining experiments. (C–D) Differences in EDA and FGF12 expression between gastric cancer tissues and normal tissues calculated using the *t*-test. (E) The correlation between EDA expression and FGF12 expression in GC tissues. (F–G) The relationship between the probability of patient survival and EDA and FGF12 expression calculated using the log-rank test.

4. Discussion

Tumor stroma, also called the TME, contains immune cells, vessels, fibroblasts and extracellular matrix, and has a significant impact in the early development of gastrointestinal cancers, growth, and dissemination. One of the most critical components of TME is CAFs, which are key regulators of both cell proliferation and tumor development physically support epithelial cells, and can both promote and retard cancer growth [25]. EDA is involved in human ectoderm-derived organs, inflammation, immune responses, and various signaling pathways. In this study, we observed that EDA expression was associated with poor prognosis in GC and drug sensitivity of 5-fluoropyrimidine. Meanwhile, expression of EDA was higher in GC than that in normal group. Through single cell analysis and IHC experiments, we observed a significant positive correlation with CAFs marker FGF12 in GC. From the above data analysis, we suggest EDA may be a novel marker of cancer fibroblasts in stomach cancer.

Recent research has found that TGFβ is able to induce EDA, which binds to TGF-β1 and triggers the enhancement of α-SMA (α-actinin-2) and fibroblast contraction, thereby inducing fibroblast and ECM contraction function [26]. Activation of Wnt signaling can be achieved by the EDA-EDAR pathway, which serves as a canonical effector of cell development and cancer progression. The Wnt/β-catenin pathway is important to colorectal cancer initiation. EDAR abnormality promotes tumorigenesis by both increasing
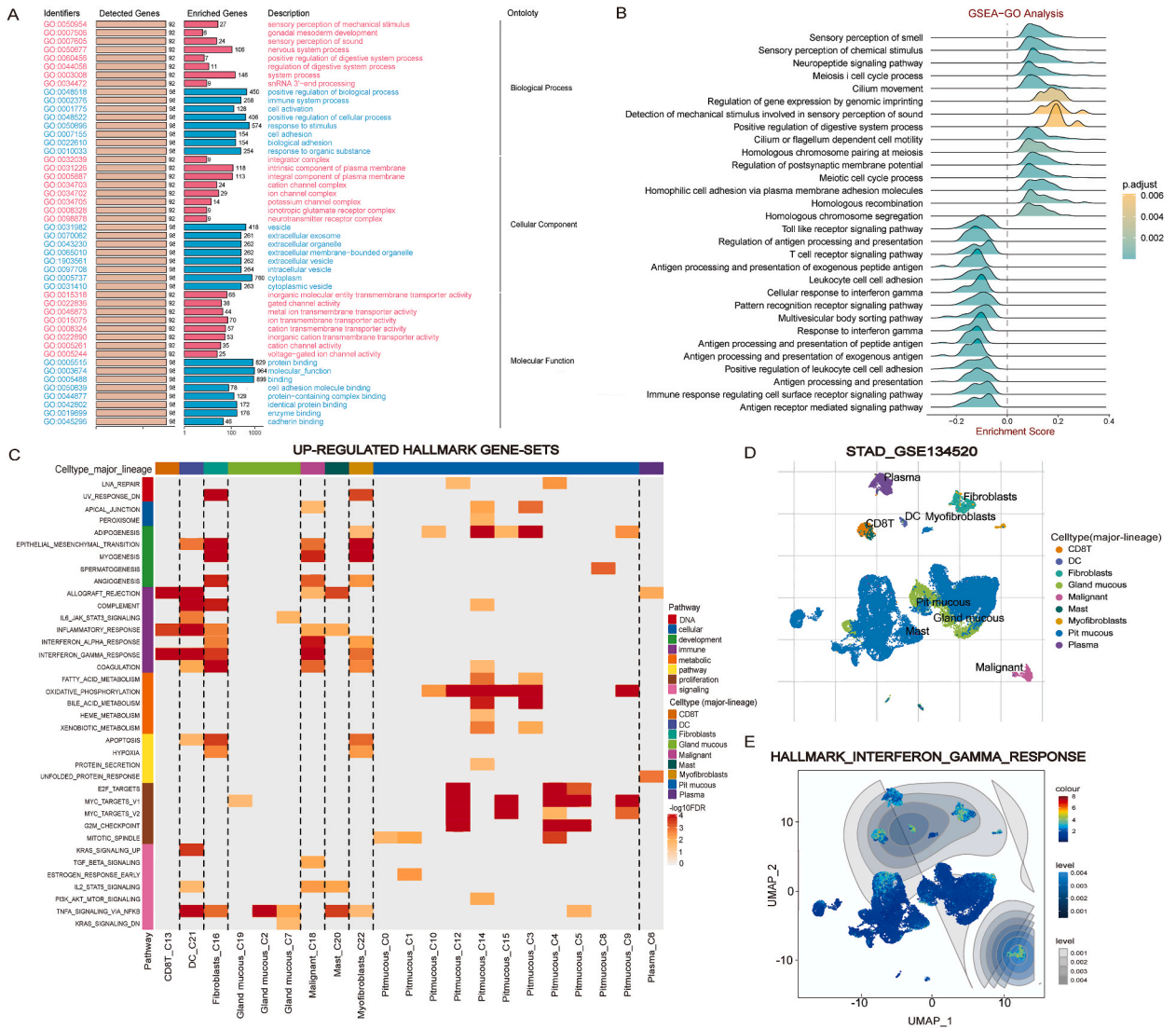
Fig. 5. Gene enrichment analysis. (A) GO enrichment analysis was conducted based on the top 1000 genes using the BEST database. (B) GSEA enrichment analysis was performed using the BEST database. (C) Single-cell hallmark enrichment analysis was conducted using the TISCH database to explore the function of EDA at single-cell resolution. (D–E) The TISCH database's single-cell signature explorer was used to analyze the EDA enrichment pathways in different cell types.

β-catenin and c-Myc and encouraging tumor cell proliferation via reducing crucial elements within cellular cycle checkpoints [27]. Through correlation analysis, we revealed that EDA positively corelated with various CAF marker genes, especially FGF12. The fibroblast growth factor (FGF) is a pleiotropic protein in cells that exerts autocrine, endocrine, and paracrine functions by activating four tyrosine kinases, thereby triggering a variety of cellular processes including angiogenesis, apoptosis evasion and homeostasis *in vivo*. In tumors, the four main mechanisms of angiogenesis, inflammation, cell proliferation and metastasis are more actively affected by FGF and FGF receptor (FGFR).

Although small molecules and antibody against FGFR signaling have large potential in cancer therapeutics, but how to solve resistance to drugs, activation of the alternative pathway, and the systemic toxic side effect of the modulator are key to the clinical efficacy of anti-FGFR. FGF12 can rapidly internalized into the cell by two cell-penetrating peptide (CPP) structures (CPP-M, CPP-C) in a manner independent of FGFR [28]. In addition, FGF12 derived from the tumor can activate CAF, which in turn sustains cancer proliferation and metastasis [29]. In our study, it can be concluded that high expression of EDA correlates with poor prognosis in gastric cancer patients. High correlation between EDA and FGF12 suggests EDA may stimulate fibroblast synthesis and activate CAF to affect cancer through FGF12, which need further experiments to examine whether this hypothesis makes sense.
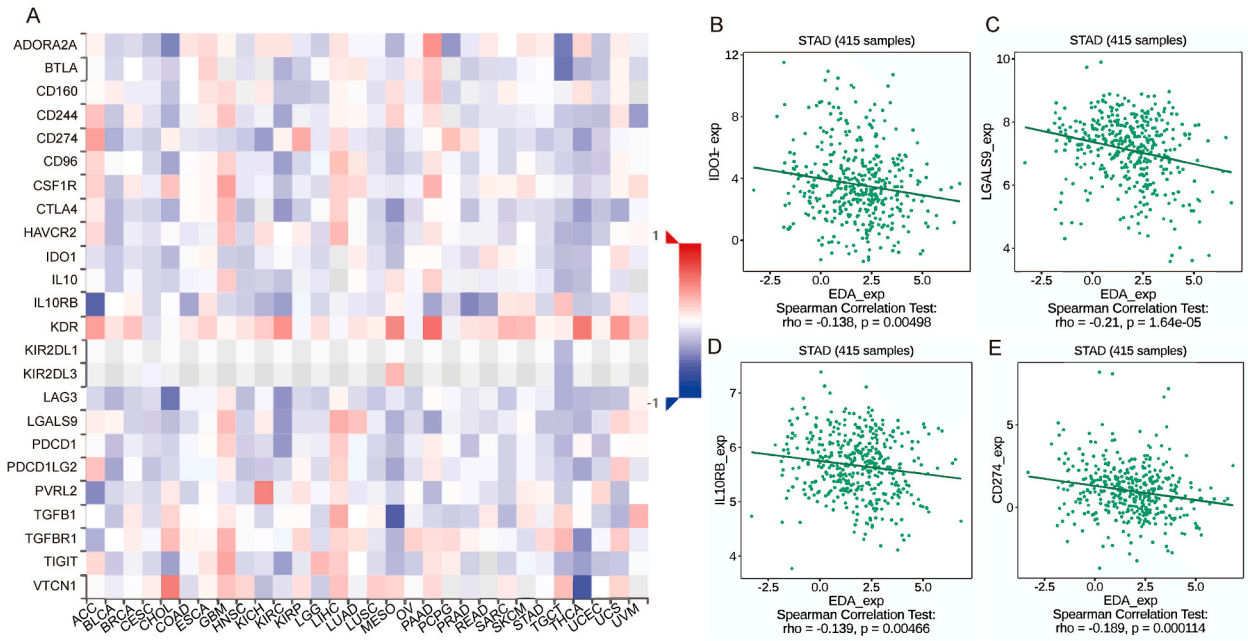
Fig. 6. The link between the EDA expression and immune inhibitors in GC. (A) The TISIDB database was applied to explore the relationship between EDA and immune inhibitors expression across different cancers. (B–E) The TISIDB database was applied to explore the correlation between EDA expression and immune inhibitors expression by spearman correlation test.

5. Conclusion

In summary, this paper indicated that EDA expression is significantly positively associated with the CAF marker FGF12 in GC patients. The higher expression of EDA leads to shorter FP, OS, and PPS times, while its impact on cancer progression and response to chemotherapy targeting EDA gene needs further exploration. GO/GSEA enrichment and single-cell data analysis illustrate an association between EDA and immune related pathway, especially interferon-gamma response. Besides perioperative chemotherapy, immunotherapy has been illustrated to have an important effect from third-line treatment to first-line treatment in GC patients [30]. We revealed that expression of EDA was negatively related to indicated immune checkpoints, suggesting that EDA may be a new biomarker or target for immunotherapy focusing on its effect on cancer-associated fibroblasts.

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Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Ya Zhang: Writing – original draft, Visualization, Conceptualization. **Haoran Chen:** Software, Methodology, Data curation. **Wenzheng Zhang:** Software, Data curation. **Haiyan Zhou:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34005.

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