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Single-cell RNA sequencing and spatial transcriptomics reveal the heterogeneity and intercellular communication of cancer-associated fibroblasts in gastric cancer

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Abstract

Background Gastric cancer is a highly aggressive malignancy characterized by a complex tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs), which are a key component of the TME, exhibit significant heterogeneity and play crucial roles in tumor progression. Therefore, a comprehensive understanding of CAFs is essential for developing novel therapeutic strategies for gastric cancer.

Methods This study investigates the characteristics and functional information of CAF subtypes and explores the intercellular communication between CAFs and malignant epithelial cells (ECs) in gastric cancer by analyzing single-cell sequencing data from 24 gastric cancer samples. CellChat was employed to map intercellular communication, and Seurat was used to integrate single-cell sequencing data with spatial transcriptome data to reconstruct a comprehensive single-cell spatial map. The spatial relationship between apCAFs and cancer cells was analyzed using multicolor immunohistochemistry.

Results Cells were categorized into nine distinct categories, revealing a positive correlation between the proportions of epithelial cells (ECs) and fibroblasts. Furthermore, six fibroblast subpopulations were identified: inflammatory (iCAFs), pericytes, matrix (mCAFs), antigen-presenting (apCAFs), smooth muscle cells (SMCs), and proliferative CAFs (pCAFs). Each of these subpopulations was linked to various biological processes and immune responses. Malignant ECs exhibited heightened intercellular communication, particularly with CAF subpopulations, through specific ligand-receptor interactions. High-density regions of CAF subpopulations displayed spatial exclusivity, with pericytes serving as a source for iCAFs, mCAFs, and apCAFs. Notably, malignant ECs and apCAFs showed increased interactions, with certain ligand-receptor pairs potentially impacting the prognosis of gastric cancer. Multiplex immunohistochemistry (mIHC) confirmed the close spatial proximity of apCAFs to cancer cells in gastric cancer.

Conclusion Our study provided a comprehensive characterization of CAF heterogeneity in gastric cancer and revealed the intricate intercellular networks within the TME. The identified CAF subpopulations and their interactions with malignant cells could serve as potential therapeutic targets.

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Highlights

1. Six distinct fibroblast subpopulations with unique roles in tumor progression and immune response were identified in gastric cancer.
2. Increased interactions between malignant epithelial cells and CAFs, highlighted the importance of specific ligand-receptor pairs in gastric cancer.
3. ApCAFs were in close spatial proximity to cancer cells in gastric cancer.

Keywords Gastric cancer, Cancer-associated fibroblasts, Single-cell transcriptomics, Cell–cell interactions, Tumor microenvironment

Introduction

Gastric cancer is the fifth most frequently diagnosed cancer and the fifth leading cause of cancer death globally, responsible for almost 0.97 million new cases and 0.66 million deaths according to Global Cancer Statistics 2022 [1]. Despite recent advances in the diagnosis and treatment of gastric cancer, its prognosis remains poor [2–5]. The development and progression of gastric cancer is a complex, multi-stage process involving the interaction of various cell types [6–9] and molecular mechanisms [10–12]. Among these, the tumor microenvironment (TME) plays a crucial role in the progression of gastric cancer [13]. The TME is composed of a variety of cells, including tumor cells, immune cells, fibroblasts, endothelial cells, and others [14]. These cells interact through complex signaling pathways, collectively influencing tumor growth, invasion, and metastasis.

In recent years, the rapid development of single-cell transcriptomics has provided unprecedented opportunities for in-depth analysis of the heterogeneity within the TME. Single-cell gene expression studies have revealed transcriptional heterogeneity and extensive reprogramming of cells within the gastric TME [15]. Additionally, scRNA-seq studies have provided a comprehensive map of gastric cancer metastasis and identified several metastasis marker genes as well as potential evolution-driving genes in gastric cancer, providing a basis for the treatment of gastric cancer [16]. Despite the valuable insights into the cellular composition and function of the TME provided by single-cell transcriptomics, it does not offer spatial location information of cells. Spatially resolved transcriptomics addresses this limitation by supplying spatial information that is essential for comprehending cell–cell interactions and the organization of TME [17–19]. Therefore, integrating single-cell transcriptomics with spatial information to comprehensively analyze the complexity of the TME represents an important gap in the current research field.

Cancer-associated fibroblasts (CAFs) are the predominant stromal cell type within TME and exhibit distinct tumorigenic properties [20]. Research has shown that CAFs facilitate proliferation, angiogenesis, metastasis, and immunosuppression in gastric cancer [21]. The interaction between CAFs and the TME is a critical factor influencing tumor progression and the prognosis of gastric cancer [8]. Although scRNA-seq provides an unprecedented opportunity to systematically analyze the heterogeneity of CAFs, the lack of spatial information during tissue dissociation restricts the exploration of interactions between CAFs and the TME. Furthermore, the roles of CAFs in the TME may vary significantly depending on their subtypes, however, systematic research on the spatial distribution of CAF subtypes and their interactions with tumor cells remains limited [22, 23]. Therefore, an in-depth analysis of the spatial characteristics of CAF subtypes and their mechanisms of action within the TME is of great significance for understanding the biological behavior of gastric cancer and identifying new therapeutic targets.

This study aims to comprehensively analyze the cellular heterogeneity and spatial distribution characteristics of the gastric cancer microenvironment by integrating single-cell and spatial transcriptomics technologies. We used scRNA-seq data to finely cluster cell types in the gastric cancer microenvironment and combined spatial transcriptomics data to reveal the spatial locations and interactions of cell subpopulations. Specifically, we focused on the subtypes of CAFs and their interaction mechanisms with tumor cells. Through this multidimensional analytical approach, our study not only fills the gap in spatial information of cells in the gastric cancer microenvironment but also provides new insights into the mechanisms of CAFs in gastric cancer progression, potentially offering new theoretical bases and potential targets for the diagnosis and treatment of gastric cancer.

Materials and methods

Data collection

The gastric cancer-related scRNA-seq data sets CRA002586 (n = 9), PRJNA776683 (n = 2), and

HRA003647 (n = 13) were downloaded from the Genome Sequence Archive (GSA, https://ngdc.cncb.ac.cn/gsa) database. Additionally, the gastric cancer-related spatial transcriptome data set GSE251950 (n = 9) was obtained from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database.

Moreover, the transcriptomic data and the corresponding clinical information of gastric cancer patients were collected from the Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/) and GEO databases. This included the gastric cancer prognosis modeling cohort (TCGA, n = 386) and three validation cohorts (GSE62254, n = 300; GSE84437, n = 433; GSE26901, n = 109).

Quality control and cell annotation of scRNA-seq data

Cell Ranger V6.1.2 was utilized to align the original reads to the human genome (GRCh38), while Seurat V4.1.1 was employed for processing scRNA-seq data. Cells exhibiting more than 10% mitochondrial content, over 5% hemoglobin content, fewer than 200 expressed genes, or more than 5,000 expressed genes were excluded from the analysis. Data normalization, cell clustering, and dimensionality reduction were conducted using the Seurat package. The "FindVariableFeatures" function was implemented to identify 2000 highly variable genes from the corrected expression matrix. Subsequently, principal component analysis (PCA) was performed using the "RunPCA" function, retaining the top 20 principal components for further analysis. Batch effects were mitigated employing the "RunHarmony" function from the Harmony R package. Cell clustering (resolution 0.9) was performed using the “FindClusters” function, and nonlinear dimensionality reduction was performed using “RunUMAP” function. Common marker genes were used to annotate the cell clusters based on the “SingleR” package.

Identifying and functional enrichment of differentially expressed genes (DEGs)

(DEGs between the various groups were identified using the 'FindMarkers' function from the 'Seurat' package. DEGs were selected based on an adjusted p-value of < 0.05 and an average fold change greater than 0.3. Furthermore, Gene Ontology (GO) analysis, encompassing Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, were conducted using the 'clusterProfiler' package in R. Significantly enriched GO terms and KEGG pathways were determined with an adjusted p-value of < 0.05, and the top 30 GO terms and 20 KEGG pathways were presented.

Identification of malignant epithelial cells (ECs)

Using single-cell gene expression and chromosome sequencing data, malignant ECs and non-malignant ECs were distinguished employing the 'inferCNV' v1.6.0 package in R. The settings for the inferCNV analysis included a cutoff of 0.1, clustering by groups set to TRUE, denoising enabled, and the hidden Markov model (HMM) activated. To reduce false positives in copy number variation (CNV) inference, the default Bayesian latent mixture model was utilized to evaluate the posterior probability of variants in each cell, applying a threshold of 0.5. Subsequently, the CNV scores of all genes were hierarchically clustered based on ECs and reference cells (T cells) using the k-means algorithm. ECs exhibiting low CNV scores and clustering with reference cells were classified as non-malignant, whereas those with elevated scores were designated as malignant ECs.

Spatial annotation

The gene-spot matrices generated following the processing of spatial transcriptomics data were analyzed using the Seurat package. The normalization of spots was performed utilizing the 'SCTransform' function. Single-cell datasets from CRA002586 (9 tumors), HRA003647 (13 tumors), and PRJNA776683 (2 tumors) were integrated and cell annotations were conducted using the 'FindTransferAnchors' function.

Trajectory analysis

Trajectory analysis was conducted utilizing the "Monocle 2" package to elucidate the differentiation trajectories of CAFs. An integrated expression matrix, from which batch effects had been removed, served as the input data. Unit trajectories and evolutionary orders were inferred based on the default parameters. The identification of highly variable genes associated with cell trajectories was achieved through the application of the "graph_test" function.

Cell–cell interactions

The "CellChat" package was utilized to predict and visualize biologically significant cell-to-cell communications. Specifically, the "createCellChat" function was employed to generate a CellChat object, which was subsequently annotated with labels and identified overexpressed genes. The communication probabilities were inferred using the "computeCommunProb" function, while the intercellular communication for each specific cell signaling pathway was predicted through the "computeCommunProbPathway" function.

Survival analysis

Gastric cancer patients were divided into high- and low-risk groups. Kaplan–Meier (KM) survival analysis was used to evaluate the overall survival (OS) of patients in the high- and low-risk groups and the two-sided log-rank test was used for comparison.

Multiple immunofluorescence (mIHC) staining

Gastric cancer tissues were obtained from the affiliated cancer hospital of Zhengzhou university. Detailed patient information is shown in Table S1. Anti-PDGFRα (Cell Signaling, CST5241T, 1: 200), Anti-CD74 (Cell Signaling, CST77274T, 1: 500), and PanCK (ZSGB, ZM0069, 1: 200) were used as primary antibodies in this study.

mIHC staining was conducted using the AlphaTSA Multiplex IHC Kit (AXT37100031, Alphaxbio) in accordance with the manufacturer's instructions. The tissue chip underwent dewaxing followed by hydration through a series of washes with xylene and alcohol. Subsequently, antigen retrieval and sealing procedures were performed. The sections were blocked and incubated with both primary and secondary antibodies, followed by fluorescent staining. Finally, cell nuclei were counterstained with DAPI for 5 min and mounted in Mounting Medium. Film reading was executed using ZEN (v3.1) software.

Statistical analysis

The independent samples Mann–Whitney U test was applied to continuous variables, and the chi-square test was applied to categorical variables for comparison between two groups. All statistical analysis in this study was performed with R software (v 4.0.5). Two-tailed $P < 0.05$ were considered statistically significant.

Results

Single-cell transcriptomics revealed distinct cellular compositions in gastric cancer samples

A total of 116,443 cells from 24 gastric cancer samples were clustered and categorized into 31 distinct clusters (Fig. 1A, B). These clusters were annotated into nine categories based on specific marker genes: T cells (CD3D, CD3E), plasma cells (IGHA1, IGHG1), B cells (CD79A, MS4A1), ECs (EPCAM, KRT19), fibroblasts (COL1A2, DCN), myeloid cells (MRC1, CD163), endothelial cells (PECAM1, VMF), proliferative cells (TOP2A, CENPF), and mast cells (KIT, TPSB2) (Fig. 1C, D). Of note, gastric cancer samples exhibiting a high proportion of ECs corresponded with a low proportion of fibroblasts, and vice versa (Fig. 1E). Gastric cancer is a malignant tumor that originates from gastric mucosal ECs [24]. Consequently, we calculated the CNV scores for 12,912 ECs in gastric cancer, identifying a total of 9,792 malignant ECs and 3,120 non-malignant ECs (Fig. 1F). As shown in Fig. 1E and G, gastric cancer samples with a higher proportion of fibroblasts also demonstrated a higher proportion of malignant ECs. Therefore, we subsequently concentrated on characterizing fibroblasts and ECs in this study.

Subclustering and annotation of fibroblasts

We re-clustered a total of 17,144 fibroblasts and identified six distinct cell types based on their marker gene expression: inflammatory CAFs (iCAFs, CFD), pericytes (FRZB), matrix CAFs (mCAFs, POSTN), antigen-presenting CAFs (apCAFs, HLA-DRB1), smooth muscle cells (SMCs, MYH11), and proliferative CAFs (pCAFs, TOP2A) (Fig. 2A–C). Notably, Gastric cancer samples characterized by a high proportion of malignant ECs, such as tumor1, tumor4, and tumor8, exhibited an increased prevalence of iCAFs, apCAFs, and pericyte subgroups (Fig. 2D). We identified DEGs across all six fibroblast subpopulations to elucidate the different roles of the four fibroblast subpopulations (Fig. 2E). GO and KEGG enrichment analyses revealed that genes with elevated expression levels in fibroblast subpopulations were associated with responses to mechanical stimulation, reactive oxygen species, epithelial cell proliferation, immune system activity, and cell migration (Fig. S1). Subsequently, we investigated the heterogeneity of CAFs using the AUCell algorithm, which was based on the functional characteristics of CAFs summarized by Lavie et al. [25] (Fig. 2F). iCAFs exhibited the highest activity in immune-related functions, including complement activation, chemokine production, and inflammatory responses (Fig. 2F). Conversely, apCAFs were significantly enriched in biological processes of interferon type I (IFN-I) production and chemokine production (Fig. 2F). Furthermore, mCAFs demonstrated enrichment in biological processes such as angiogenesis, wound healing, extracellular matrix (ECM) tissue regulation, and collagen biosynthesis (Fig. 2F). Interestingly, in addition to their role involvement in the cell cycle, pCAFs also participate in IFN-I production (Fig. 2F). Transcription factor analysis results revealed that the key regulatory transcription factors for mCAFs, iCAFs, pCAFs, and apCAFs in gastric cancer were ETV4, MYB, E2F8, and ETV5, respectively (Fig. 2G).

The complexity of CAF properties can be attributed to their highly heterogeneous origins [26]. In addition to their transformation from tissue-resident fibroblasts, pericytes represented another significant source of CAFs. Through trajectory analysis, a potential transformation pathway from pericytes to iCAFs, mCAFs, apCAFs, and pCAFs was proposed (Fig. 2H). CAF subpopulations exhibited distinct differentiation patterns and transcriptional repression. Cell fate 1 primarily expressed genes associated with epithelial-mesenchymal transition, while

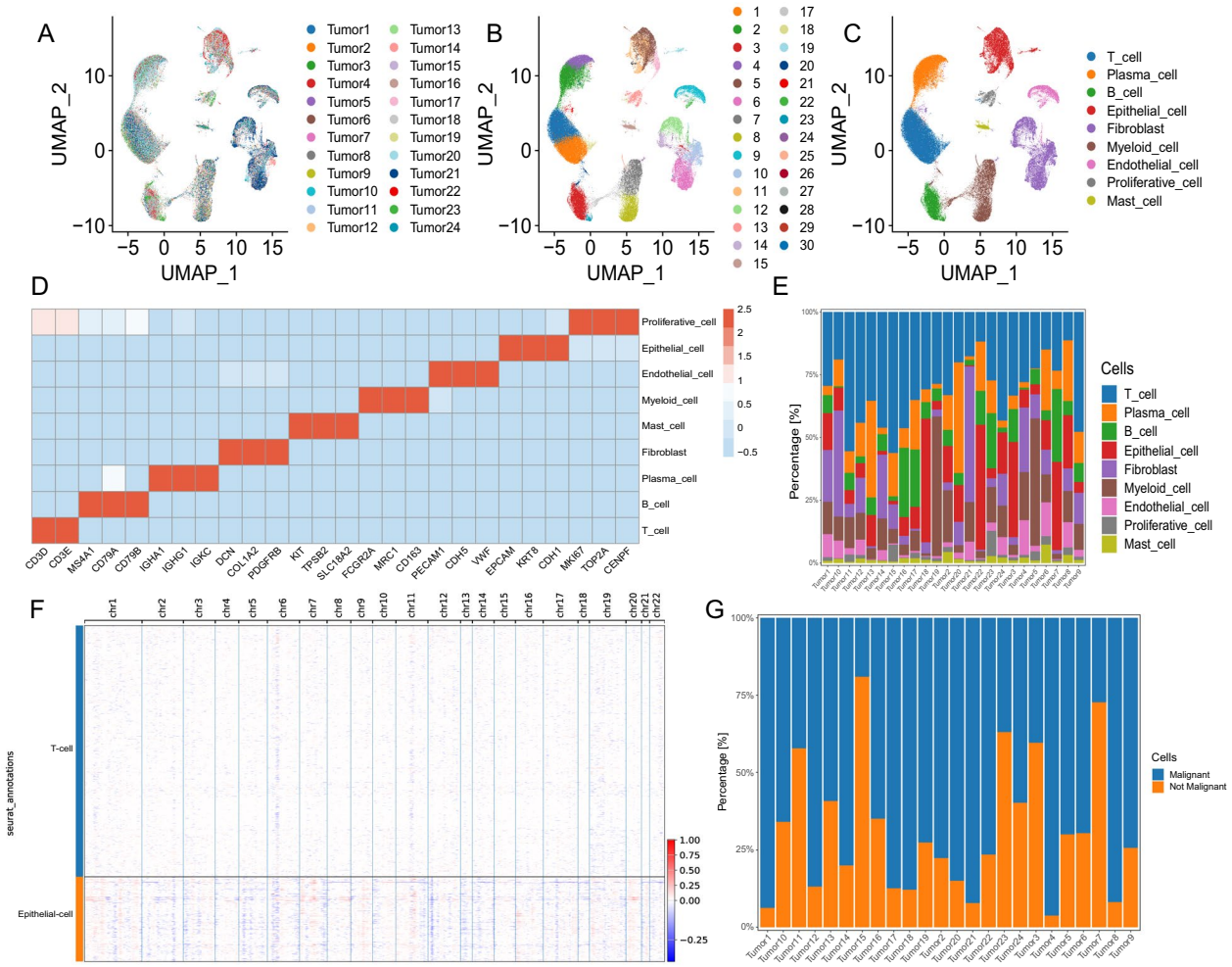

Fig. 1 Single-cell transcriptomics revealed distinct cellular compositions in gastric cancer samples. **A** UMAP diagram shows the source of cells. **B** Cell grouping results. **C** Cell annotation results. **D** Marker gene expression. **E** Proportion of cells in each sample. **F** InferCNV results. **G** Proportion of malignant cells in each sample

cell fate 2 not only showed high expression of epithelial-mesenchymal transition-related genes but also significantly expressed genes related to the inflammatory response (Fig. S2). These differences may be attributed to their complex origins.

Cell–cell interactions

CellChat was utilized to delineate the intricate intercellular networks derived from scRNA-seq. Figure 3A and B showed the intercellular communication among cell populations in gastric cancer samples. This communication predominantly involved iCAFs, malignant epithelial cells, mCAFs, and pCAFs, each exhibiting distinct numbers and strengths of interactions. Notably, the quantity and intensity of interactions between malignant ECs and surrounding cells were significantly greater than those observed in normal ECs. CellChat can predict the afferent (receptor) and efferent (ligand) activities of cell signaling pathways using scRNA-seq data, while accounting for the multimeric structure of the ligand-receptor complex and the interactions of cofactors with ligand-receptor dynamics. The predicted afferent and efferent pathway signals are presented for each cell population. The overlap of efferent and incoming signals from a pathway, either within or between groups, indicates potential interactions through that pathway. Different cell populations exhibited varying levels of relative signal intensity in the efferent signaling pattern, as illustrated in Fig. 3C and D. Notably, malignant ECs exhibited higher levels of both efferent and afferent signaling compared to normal ECs. Furthermore, ligand-receptor pair analysis indicated that malignant ECs preferentially communicate with CAF subpopulations through MDK-NCL, MDK-SDC2, PRSS3-F2R, and MDK-LRP1, whereas normal ECs tend

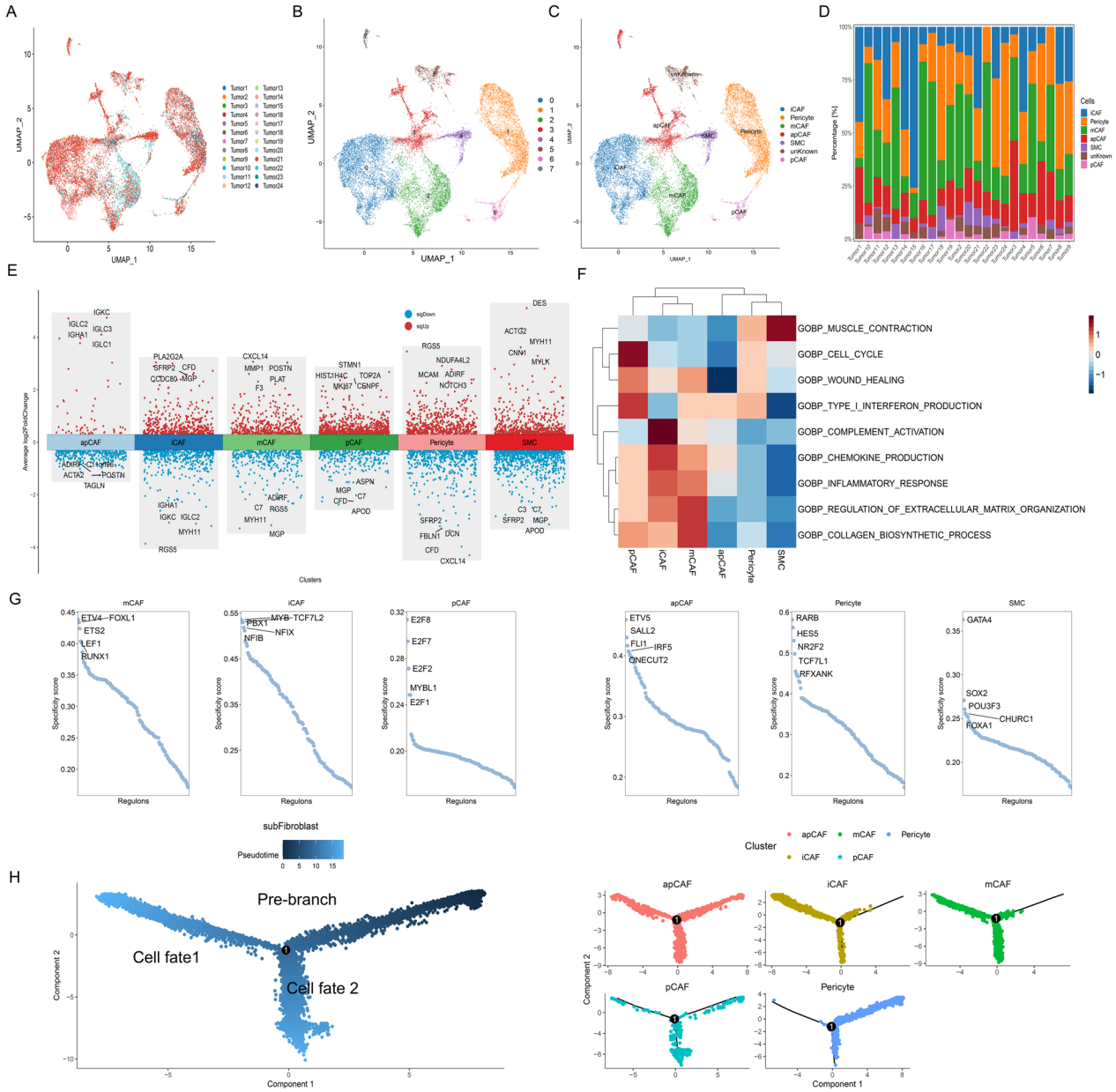

Fig. 2 Subclustering and annotation of fibroblasts. **A** UMAP diagram shows the origin of fibroblasts. **B** Cell grouping results of fibroblast subpopulations. **C** Annotation results of fibroblast subpopulation cells. **D** The proportion of fibroblast subpopulation cells in each sample. **E** The expression of marker genes in each subpopulation of fibroblasts. **F** Heatmap showing pathway activity scored by AUCell in each CAF subtype. **G** Scatter plot showing the regulon specificity score (RSS) of transcription factors in each fibroblast subtype, with the top five transcription factors highlighted. **H** Trajectory analysis results for each CAF subtype

(See figure on next page.)

Fig. 3 Cell–cell interactions. The heat map shows the number (**A**) and intensity (**B**) of interactions between cells. The vertical axis represents cells that send signals, and the horizontal axis represents cells that receive signals. The darker the color, the stronger the intensity. The heat map shows the pathways involved in cell incoming signals (**C**) and outgoing signals (**D**). The horizontal axis represents the cell type, and the vertical axis represents the signal pathway. The darker the color, the stronger the intensity of the incoming (outgoing) pathway. **E** Ligand-receptor pairs of epithelial cells to CAFs (efferent signals from epithelial cells, incoming signals from CAFs). **F** Ligand-receptor pairs from CAFs subpopulations to epithelial cells (efferent signals from CAFs, incoming signals from epithelial cells) cell incoming signal)

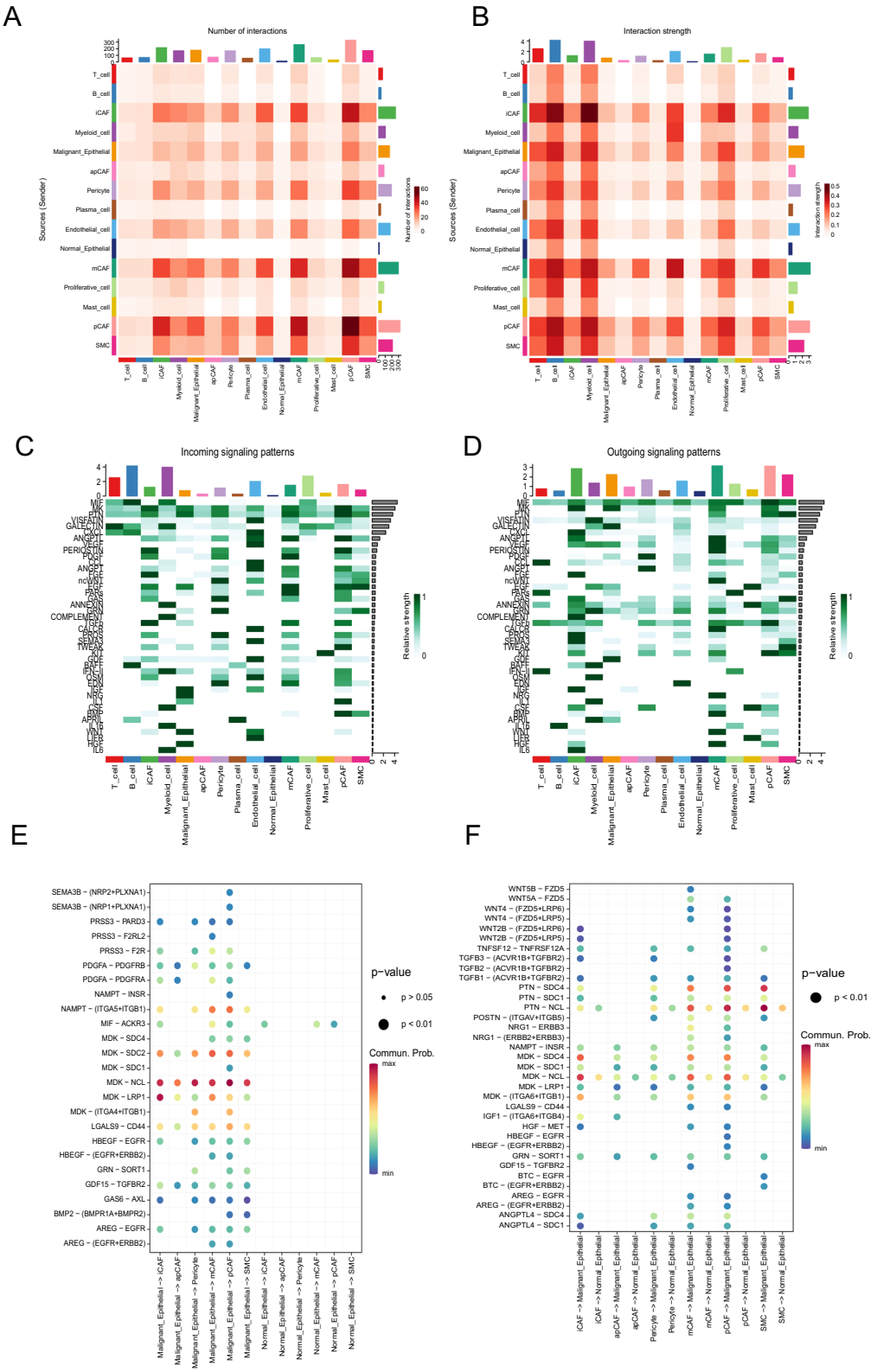

Fig. 3 (See legend on previous page.)

to communicate with a subset of CAFs via MIF-ACKR3 (Fig. 3E). Moreover, mCAFs and pCAFs preferentially utilize PTN-NCL and MDK-NCL for communication with ECs, demonstrating a stronger communication effect on malignant ECs compared to normal ECs (Fig. 3F).

Pericytes serve as a crucial source for the formation of apCAFs, mCAFs, and iCAFs

Furthermore, we utilized Seurat to integrate single-cell sequencing data with spatial transcriptome data, thereby reconstructing a spatial single-cell map. Even in the absence of corresponding scRNA-seq data from the same patient, the co-embedding results demonstrated that the spatial transcriptome dataset was largely encompassed by the scRNA-seq dataset (Fig. 4A–C).

To determine the spatial distribution characteristics of CAF subpopulations, we incorporated their cell subpopulation annotation information into the Seurat object. Our observations revealed spatial exclusivity among high-density regions of CAF subpopulations (Fig. 4D–F), indicating a correlation between the activation status of CAFs and their localization within the TME. To investigate the spatial expression dynamics of high-density regions within each CAF subpopulation, we conducted a spatial trajectory analysis across all slices (Fig. 4G–J). The findings indicated that both the cell types and their proportions along the trajectory exhibited gradual changes. This result was consistent with the temporal analysis of scRNA-seq data, which suggested that pericytes serve as a crucial source for the formation of apCAFs, mCAFs, and iCAFs (Fig. 4J).

Spatial cell interactions

Given the low cell ratios of mCAFs, iCAFs, and pCAFs, we concentrated our analysis on the interactions between malignant tumor cells and apCAFs within the TME (Fig. 5A). The results indicated an increase in the number of interactions between malignant ECs and apCAFs, with 7 out of 9 sections demonstrating such interactions (Fig. 5B). Further analysis revealed ligand-receptor interactions (LRIs) between malignant cells and apCAFs, such as PRSS3-F2R, MDK-LRP1, and SEMA3B-NRP2-PLXNA3, etc. (Fig. 5B, C). Functional enrichment results indicated that these ligands play significant roles in cell growth, cytokine binding, complement, and inflammatory pathways (Fig. 5D–F). We found that F2R, NRP1, NRP2, and SDC2 were significantly differentially expressed between tumor and normal samples, and these four genes correlated with the prognosis of gastric cancer patients. Furthermore, we constructed a risk score model using these four genes: Risk score = 0.005001 × F2R + 0.035099 × NRP1 + 0.008414 × NRP2 + 0.012444 × SDC2. Compared to the low-risk group, the high-risk group was correlated with a poor prognosis of gastric cancer patients across multiple independent datasets (Fig. 5G). This suggests that certain ligand-receptor pairs may play a crucial role in the progression and prognosis of gastric cancer.

apCAFs were in close spatial proximity to cancer cells

To further elucidate the spatial relationship between apCAFs and cancer cells, we conducted an analysis of whole-tumor tissue sections using mIHC assay. apCAFs were identified as cells co-expressing CD74 and PDGFRα. Our findings revealed a significant presence of apCAFs within gastric cancer tissues, with these double-positive cells located in close proximity to cancer cells, as indicated by Pan-CK positivity (Fig. 6). These observations imply a potential interaction between apCAFs and gastric cancer cells, which may have significant implications for the TME and the immune response.

Discussion

Our study provides a comprehensive description of the heterogeneity of TME in gastric cancer, highlighting the interactions between CAF subpopulations and ECs within the TME. Notably, gastric cancer samples characterized by a higher proportion of malignant ECs also exhibited an increased proportion of iCAFs, apCAFs, and pericyte subgroups.

In previous studies, CAFs exhibit significant heterogeneity in gastric cancer, which can be classified into various subgroups, including iCAFs and mCAFs [8]. These subgroups play distinct roles in tumor progression [7, 20]. In this study, we found that in gastric cancer samples, the proportion of malignant ECs was relatively high, along with an increase in the proportion of iCAFs, apCAFs, and pericyte subpopulations. In particular, we discovered a significant presence of apCAFs in gastric cancer tissues, which were in close spatial proximity to cancer cells. To our knowledge, the role of apCAFs in gastric cancer has been infrequently reported and remains largely unexplored. ICAFs were correlated to inflammatory responses and immunosuppression in tumors, potentially facilitating the growth and invasion of gastric cancer by releasing pro-inflammatory cytokines and chemokines [27]. Additionally, in various solid tumors, including gastric cancer, ICAFs might chemotactically draw T cells and modulate their activities through the secretion of IL6 and CXCL12 [8, 28, 29]. Peng et al. identified two differentiation states of iCAFs (iCAFs-S1, iCAFs-S2) present in different tissues of gastric cancer, and iCAFs-S1 expressed high levels of immuno-inflammatory genes, whereas iCAFs-S2 were strongly associated with ECM remodeling [30]. Our investigations also revealed that iCAFs exhibited heightened activity related to complement activation,

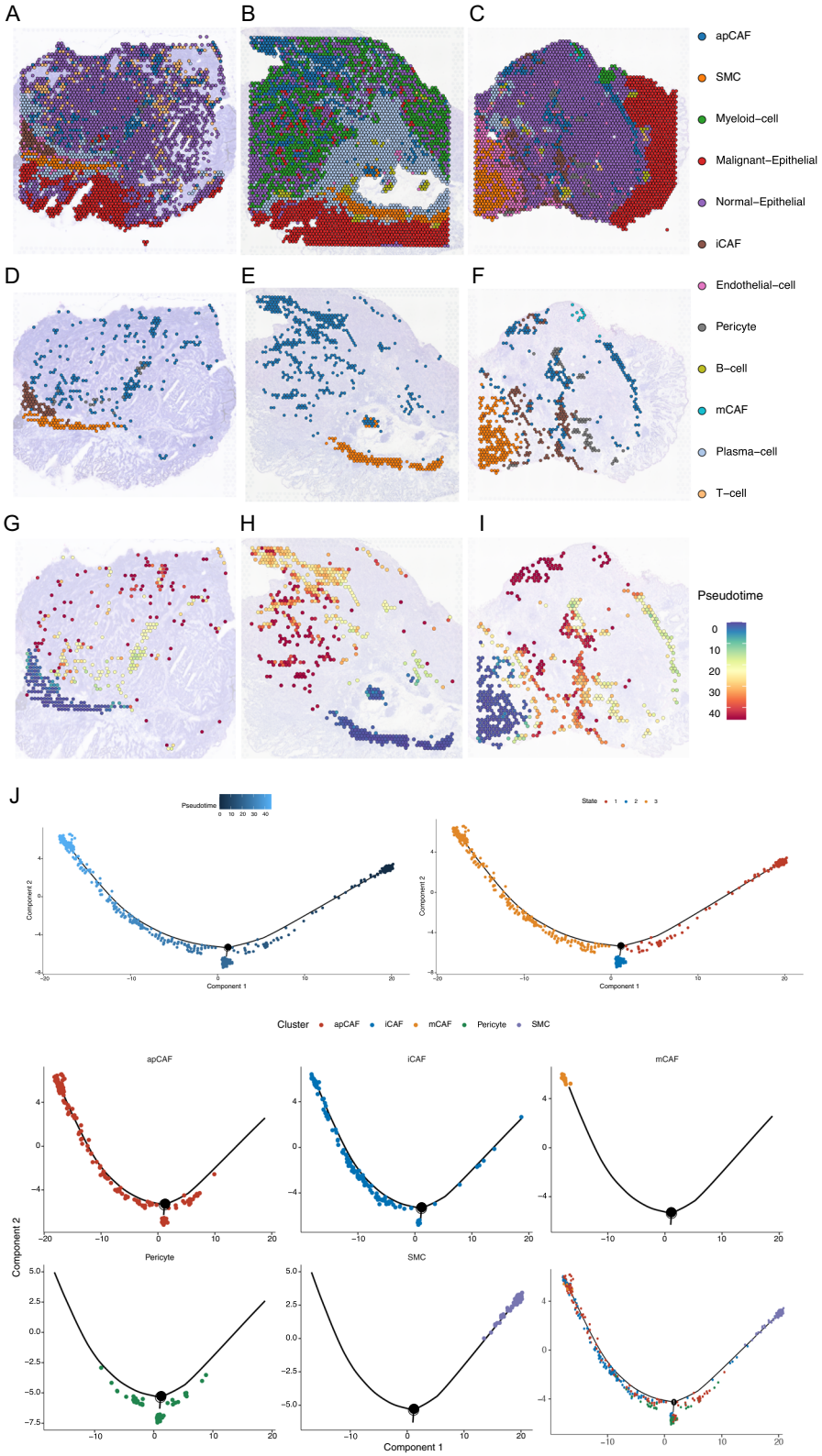

Fig. 4 Spatial annotation and trajectory analysis results of gastric cancer tissue sections. **A**–**C**, Spatial annotation results of gastric cancer tissue sections. **D**–**F**, Spatial distribution characteristics of CAFs subpopulations. **G**–**I**, Spatial distribution characteristics of pseudo-timeline analysis of CAFs subpopulations. **J** Results of pseudo-time analysis of CAFs subpopulations in spatial slices

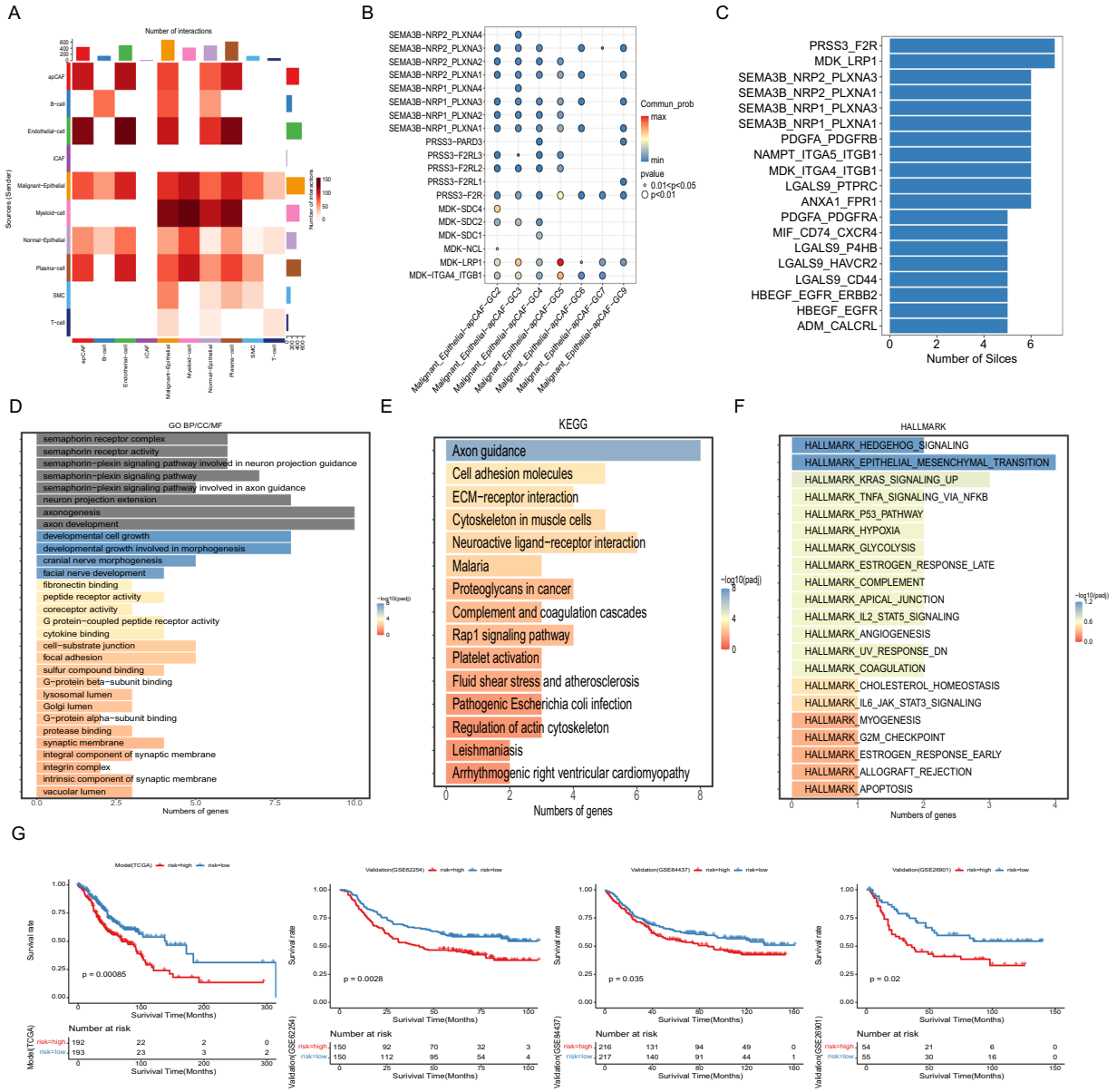

Fig. 5 Effects of CAFs on the tumor microenvironment through paracrine signaling. **A** Heat map shows the interaction between cells in the spatial slice of gastric cancer2 sample. **B** Key ligand-receptor pairs of malignant cells and apCAFs, the abscissa is the corresponding slice information. **C** Integrated ranking of ligand-receptor interactions (LRIs) from malignant epithelial cells to apCAFs in 9 tissue sections. The results of GO (**D**), KEGG (**E**), and Hallmark (**F**) enrichment analysis. **G** The overall survival rate of gastric cancer patients in the low and high risk groups

chemokine production, and inflammatory responses in the context of gastric cancer. Conversely, apCAFs were associated with antigen presentation and were likely to play a critical role in shaping immune responses within the TME [31]. It has been proposed that apCAFs may induce T cell anergy or facilitate their differentiation into regulatory T cells in pancreatic ductal adenocarcinoma and breast cancer [32, 33]. These findings suggest that iCAFs promote tumor growth and invasion in gastric cancer by enhancing inflammatory responses and attracting regulatory T cells. In contrast, apCAFs modulate the immune response within the TME by participating in antigen presentation and influencing T cell function. Pericytes, which are vital components of blood vessels, play a role in tumor angiogenesis and the stabilization of microvessels [34, 35]. Recent studies have indicated that activated fibroblasts may transdifferentiate into pericytes within the TME [36]. We identified potential translational

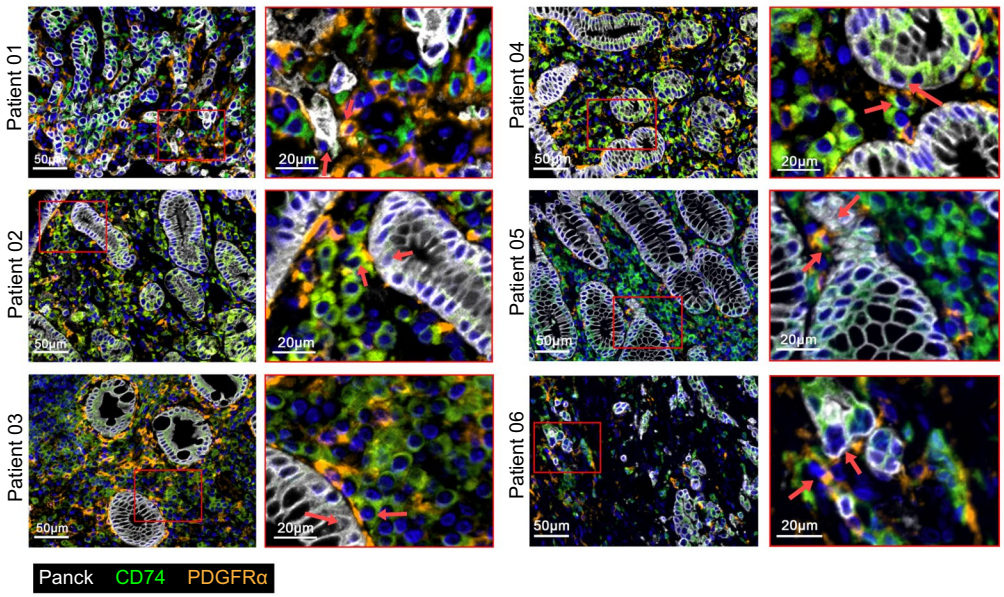

Fig. 6 Multiple immunofluorescence revealed the spatial proximity of apCAFs (double-positive for CD74 and PDGFRα) and tumor cells (Pan-CK)

pathways linking pericytes to iCAFs, mCAFs, apCAFs, and pCAFs. Nevertheless, the origins of these pericytes detected in gastric cancer require further investigation.

Transcription factor analysis results indicated that the key regulatory transcription factors for mCAFs, iCAFs, pCAFs, and ApCAFs in gastric cancer were ETV4, MYB, E2F8, and ETV5, respectively. ETV4 and ETV5 are part of the ETS transcription factors belonging to the PEA3 group. Both ETV4 and ETV5 have been implicated in the proliferation of embryonic stem cells and the induction of differentiation-associated genes in these cells [37]. Notably, ETV4 expression is significantly elevated in gastric cancer tissues, correlating with lymph node or distant metastasis and poor prognosis. Furthermore, ETV4 may promote gastric cancer cell metastasis by modulating KDM5D [38]. KIF2A, which is upregulated by the transcription factor ETV4, plays a crucial role in gastric cancer cell proliferation; its knockdown effectively inhibits proliferation and induces apoptosis through the AKT signaling pathway, suggesting that targeting KIF2A expression could be a viable therapeutic strategy for gastric cancer [39]. MYB is a critical transcription factor involved in regulating hematopoiesis and exhibits significant potency in T-cell activation. High MYB expression is positively correlated with poor overall survival in gastric cancer patients, and activated CD4+T cell infiltration is also positively associated with MYB expression in this context [40]. Additionally, MYB may influence the differentiation and immune homeostasis of effector regulatory T cells [41] and regulate CD8+T cell stemness and anti-tumor responses [42]. Accordingly, CAF transcription factors may influence the activity and function of immune cells within the TME of gastric cancer, thereby impacting the progression of the disease and the prognosis for patients.

Compared to normal fibroblasts, CAFs typically accumulate in tumor tissues and can be activated through interactions with tumor cells or immune cells within the TME [43]. Once activated, fibroblasts can engage with cancer cells, thereby promoting tumor progression [44]. In the present study, we found apCAFs interacted with malignant ECs through multiple ligands and receptors in gastric cancer. Further analysis found that four ligands or receptors (F2R, NRP1, NRP2, and SDC2) were significantly correlated with the prognosis of gastric cancer patients, and were used to construct a risk score model. Gastric cancer patients with high-risk scores were correlated with a poor prognosis, suggesting that the risk score model developed using apCAF and the ligands and receptors of malignant ECs might effectively predict the prognosis of gastric cancer patients, thereby offering valuable guidance for clinical treatment. A substantial number of apCAFs were identified in gastric cancer tissues, exhibiting close spatial proximity to cancer cells. This observation suggested intricate interactions and potential mechanisms of immune evasion within the gastric

cancer TME. Notably, apCAFs directly ligate and induce naive CD4 + T cells to differentiate into Tregs in an antigen-specific manner [45]. Tregs facilitate tumor progression and dissemination by exerting immunosuppressive effects within the tumor, which diminishes anti-tumor immunity and contributes to tumor immune evasion [46]. Consequently, we hypothesized that the complex interactions among apCAFs, malignant ECs, and Tregs might play a significant role in the progression of gastric cancer. This hypothesis requires validation in larger cohorts.

By integrating scRNA-seq and spatial transcriptomics, this study comprehensively elucidates the heterogeneity of CAFs and their spatial distribution characteristics within the TME. Furthermore, the study intensively investigates the correlation between different CAF subpopulations and patient prognosis, providing significant theoretical support for the development of prognostic assessment and therapeutic strategies. This multidimensional analytical approach offers a more holistic research perspective for understanding the functions and mechanisms of CAFs in the progression of gastric cancer. However, there were some limitations of our study. Firstly, the relatively small sample size may restrict the generalizability and extrapolation of the results. Secondly, although this study provides a detailed analysis of CAFs, it may not fully encapsulate all interactions and mechanisms within the TME. Furthermore, while the study reveals potential interactions between CAF subpopulations and malignant ECs, these findings necessitate further validation through in vitro and in vivo experiments to confirm their biological functions and clinical relevance. Lastly, the proposed CAF subpopulations and specific ligand-receptor pairs as potential therapeutic targets require validation for clinical efficacy and safety through clinical trials.

Conclusion

In conclusion, this study provides a detailed characterization of the cellular heterogeneity and spatial organization within gastric cancer, revealing the classification, potential function, origin, and interaction with malignant ECs of CAFs. Importantly, we found that a substantial number of apCAFs were identified in gastric cancer tissues, exhibiting close spatial proximity to cancer cells. This study elucidates the heterogeneity of CAFs and their role in gastric cancer, offering new insights into the origins of CAFs and their spatial dynamics during tumor progression. Future research should integrate multi-omics data (such as proteomics and metabolomics) to comprehensively elucidate the mechanisms of CAFs in the tumor microenvironment, thereby providing a more systematic biological basis for the development of novel therapeutic strategies.

Abbreviations

TME	Tumor microenvironment
CAFs	Cancer-associated fibroblasts
ECs	Epithelial cells
iCAFs	Inflammatory cancer-associated fibroblasts
apCAFs	Antigen-presenting cancer-associated fibroblasts
mCAFs	Matrix cancer-associated fibroblasts
pCAFs	Proliferative cancer-associated fibroblasts
SMCs	Smooth muscle cells
scRNA-seq	Single-cell RNA sequencing
mIHC	Multispectral immunohistochemical
GO	Gene ontology
KEGG	Kyoto Encyclopedia of Genes and Genome
CNV	Copy number variation
OS	Overall survival

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06376-8.

Supplementary Material 1. Table S1. Detailed patient information

Supplementary Material 2. Fig. S1. GOand KEGGenrichment analysis results of iCAFs, mCAFs, apCAFs, pCAFs, Pericyte, and SMC cells

Supplementary Material 3. Fig. S2. The expression of top 50 branching related genes in different branches according to the trajectory analysis. The pseudo-time related genes were categorized into six clusters based on their expression profiles. A functional enrichment analysis was conducted on the genes within these six clustersenrichment results, the right panel showed the Hallmark enrichment results). Notably, genes in cluster 1 significantly activated the epithelial-mesenchymal transition pathway, whereas genes in clusters 2 and 3 also significantly activated the epithelial-mesenchymal transition pathway, along with the inflammatory response pathway, among others

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Author contributions

Xijie Zhang.: wrote the original draft, review and editing; Xijie Zhang, Bo Ren and Bo Liu: Validation, Software, Formal analysis, Data curation, Conceptualization, Investigation; Rui Wang and Sen Li: Data curation, investigation and figure preparation; Yuzhou Zhao and Wence Zhou: Supervision.

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Availability of data and materials

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material. All data are available from the TCGA database (https://portal.gdc.cancer.gov/), GSA database, (https://ngdc.cncb.ac.cn/gsa) and GEO (https://www.ncbi.nlm.nih.gov/geo/) database within the article. GEO database under accession number GSE251950, GSE62254, GSE84437 and GSE26901.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of affiliated cancer hospital of Zhengzhou University (2021-KY-0012-001) and conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent before participating in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74:229–63.
2. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. Lancet. 2020;396:635–48.
3. Zeng Y, Jin RU. Molecular pathogenesis, targeted therapies, and future perspectives for gastric cancer. Semin Cancer Biol. 2022;86:566–82.
4. Guan WL, He Y, Xu RH. Gastric cancer treatment: recent progress and future perspectives. J Hematol Oncol. 2023;16:57.
5. The global burden of cancer attributable to risk factors, 2010–19: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022, 400:563–591.
6. Eum HH, Kwon M, Ryu D, Jo A, Chung W, Kim N, Hong Y, Son DS, Kim ST, Lee J, et al. Tumor-promoting macrophages prevail in malignant ascites of advanced gastric cancer. Exp Mol Med. 2020;52:1976–88.
7. Li Y, Hu X, Lin R, Zhou G, Zhao L, Zhao D, Zhang Y, Li W, Zhang Y, Ma P, et al. Single-cell landscape reveals active cell subtypes and their interaction in the tumor microenvironment of gastric cancer. Theranostics. 2022;12:3818–33.
8. Li X, Sun Z, Peng G, Xiao Y, Guo J, Wu B, Li X, Zhou W, Li J, Li Z, et al. Single-cell RNA sequencing reveals a pro-invasive cancer-associated fibroblast subgroup associated with poor clinical outcomes in patients with gastric cancer. Theranostics. 2022;12:620–38.
9. Huang Z, Wu C, Liu X, Lu S, You L, Guo F, Stalin A, Zhang J, Zhang F, Wu Z, et al. Single-cell and bulk RNA sequencing reveal malignant epithelial cell heterogeneity and prognosis signatures in gastric carcinoma. Cells. 2022. https://doi.org/10.3390/cells11162550.
10. Li P, Fang X, Huang D. Exploring m6A modifications in gastric cancer: from molecular mechanisms to clinical applications. Eur J Med Res. 2025;30:98.
11. Ding L, Dang S, Sun M, Zhou D, Sun Y, Li E, Peng S, Li J, Li G. Quercetin induces ferroptosis in gastric cancer cells by targeting SLC1A5 and regulating the p-Camk2/p-DRP1 and NRF2/GPX4 Axes. Free Radic Biol Med. 2024;213:150–63.
12. Park J, Wu J, Szkop KJ, Jeong J, Jovanovic P, Husmann D, Flores NM, Francis JW, Chen YC, Benitez AM, et al. SMYD5 methylation of rpL40 links ribosomal output to gastric cancer. Nature. 2024;632:656–63.
13. Ren L, Huang D, Liu H, Ning L, Cai P, Yu X, Zhang Y, Luo N, Lin H, Su J, Zhang Y. Applications of single-cell omics and spatial transcriptomics technologies in gastric cancer (review). Oncol Lett. 2024;27:152.
14. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008;27:5904–12.
15. Sathe A, Grimes SM, Lau BT, Chen J, Suarez C, Huang RJ, Poultsides G, Ji HP. Single-cell genomic characterization reveals the cellular reprogramming of the gastric tumor microenvironment. Clin Cancer Res. 2020;26:2640–53.
16. Wang B, Zhang Y, Qing T, Xing K, Li J, Zhen T, Zhu S, Zhan X. Comprehensive analysis of metastatic gastric cancer tumour cells using single-cell RNA-seq. Sci Rep. 2021;11:1141.
17. Tian L, Chen F, Macosko EZ. The expanding vistas of spatial transcriptomics. Nat Biotechnol. 2023;41:773–82.
18. Khaliq AM, Rajamohan M, Saeed O, Mansouri K, Adil A, Zhang C, Turk A, Carstens JL, House M, Hayat S, et al. Spatial transcriptomic analysis of primary and metastatic pancreatic cancers highlights tumor microenvironmental heterogeneity. Nat Genet. 2024;56:2455–65.
19. Zheng B, Fang L. Spatially resolved transcriptomics provide a new method for cancer research. J Exp Clin Cancer Res. 2022;41:179.
20. Zhang H, Yue X, Chen Z, Liu C, Wu W, Zhang N, Liu Z, Yang L, Jiang Q, Cheng Q, et al. Define cancer-associated fibroblasts (CAFs) in the tumor microenvironment: new opportunities in cancer immunotherapy and advances in clinical trials. Mol Cancer. 2023;22:159.
21. Li P, Zhang H, Chen T, Zhou Y, Yang J, Zhou J. Cancer-associated fibroblasts promote proliferation, angiogenesis, metastasis and immunosuppression in gastric cancer. Matrix Biol. 2024;132:59–71.
22. Biffi G, Tuveson DA. Diversity and biology of cancer-associated fibroblasts. Physiol Rev. 2021;101:147–76.
23. Li Z, Zhou J, Zhang J, Li S, Wang H, Du J. Cancer-associated fibroblasts promote PD-L1 expression in mice cancer cells via secreting CXCL5. Int J Cancer. 2019;145:1946–57.
24. Thrift AP, El-Serag HB. Burden of gastric cancer. Clin Gastroenterol Hepatol. 2020;18:534–42.
25. Lavie D, Ben-Shmuel A, Erez N, Scherz-Shouval R. Cancer-associated fibroblasts in the single-cell era. Nat Cancer. 2022;3:793–807.
26. Madar S, Goldstein I, Rotter V. 'Cancer associated fibroblasts'—more than meets the eye. Trends Mol Med. 2013;19:447–53.
27. Jiang H, Yu D, Yang P, Guo R, Kong M, Gao Y, Yu X, Lu X, Fan X. Revealing the transcriptional heterogeneity of organ-specific metastasis in human gastric cancer using single-cell RNA sequencing. Clin Transl Med. 2022;12:e730.
28. Izar B, Tirosh I, Stover EH, Wakiro I, Cuoco MS, Alter I, Rodman C, Leeson R, Su MJ, Shah P, et al. A single-cell landscape of high-grade serous ovarian cancer. Nat Med. 2020;26:1271–9.
29. Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, Corbo V, Oni TE, Hearn SA, Lee EJ, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med. 2017;214:579–96.
30. Peng Z, Fang C, Tong Z, Rao Q, Ren Z, Hu K. Crosstalk between cancer-associated fibroblasts and myeloid cells shapes the heterogeneous microenvironment of gastric cancer. Curr Genomics. 2024;25:390–411.
31. Kerdidani D, Aerakis E, Verrou KM, Angelidis I, Douka K, Maniou MA, Stamoulis P, Goudevenou K, Prados A, Tzaferis C, et al. Lung tumor MHCII immunity depends on in situ antigen presentation by fibroblasts. J Exp Med. 2022. https://doi.org/10.1084/jem.20210815.
32. Sebastian A, Hum NR, Martin KA, Gilmore SF, Peran I, Byers SW, Wheeler EK, Coleman MA, Loots GG. Single-cell transcriptomic analysis of tumor-derived fibroblasts and normal tissue-resident fibroblasts reveals fibroblast heterogeneity in breast cancer. Cancers (Basel). 2020. https://doi.org/10.3390/cancers12051307.
33. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffi G, Lucito MS, et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov. 2019;9:1102–23.
34. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and dysfunction of the blood–brain barrier. Cell. 2015;163:1064–78.
35. Stratman AN, Schwindt AE, Malotte KM, Davis GE. Endothelial-derived PDGF-BB and HB-EGF coordinately regulate pericyte recruitment during vasculogenic tube assembly and stabilization. Blood. 2010;116:4720–30.
36. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16:582–98.
37. Akagi T, Kuure S, Uranishi K, Koide H, Costantini F, Yokota T. ETS-related transcription factors ETV4 and ETV5 are involved in proliferation and induction of differentiation-associated genes in embryonic stem (ES) cells. J Biol Chem. 2015;290:22460–73.

38. Cai LS, Chen QX, Fang SY, Lian MQ, Lian MJ, Cai MZ. ETV4 promotes the progression of gastric cancer through regulating KDM5D. Eur Rev Med Pharmacol Sci. 2020;24:2442–51.
39. Zhang X, Wang Y, Liu X, Zhao A, Yang Z, Kong F, Sun L, Yu Y, Jiang L. KIF2A promotes the progression via AKT signaling pathway and is upregulated by transcription factor ETV4 in human gastric cancer. Biomed Pharmacother. 2020;125: 109840.
40. Yao F, Zhan Y, Pu Z, Lu Y, Chen J, Deng J, Wu Z, Chen B, Chen J, Tian K, et al. LncRNAs target ferroptosis-related genes and impair activation of CD4(+) T cell in gastric cancer. Front Cell Dev Biol. 2021;9: 797339.
41. Dias S, D'Amico A, Cretney E, Liao Y, Tellier J, Bruggeman C, Almeida FF, Leahy J, Belz GT, Smyth GK, et al. Effector regulatory T cell differentiation and immune homeostasis depend on the transcription factor Myb. Immunity. 2017;46:78–91.
42. Gautam S, Fioravanti J, Zhu W, Le Gall JB, Brohawn P, Lacey NE, Hu J, Hocker JD, Hawk NV, Kapoor V, et al. The transcription factor c-Myb regulates CD8(+) T cell stemness and antitumor immunity. Nat Immunol. 2019;20:337–49.
43. Huang T, Li F, Cheng X, Wang J, Zhang W, Zhang B, Tang Y, Li Q, Zhou C, Tu S. Wnt inhibition sensitizes PD-L1 blockade therapy by overcoming bone marrow-derived myofibroblasts-mediated immune resistance in tumors. Front Immunol. 2021;12: 619209.
44. Hu T, Hu J. Melanoma-derived exosomes induce reprogramming fibroblasts into cancer-associated fibroblasts via Gm26809 delivery. Cell Cycle. 2019;18:3085–94.
45. Huang H, Wang Z, Zhang Y, Pradhan RN, Ganguly D, Chandra R, Murimwa G, Wright S, Gu X, Maddipati R, et al. Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. Cancer Cell. 2022;40:656-673.e657.
46. Togashi Y, Shitara K, Nishikawa H. Regulatory T cells in cancer immunosuppression—implications for anticancer therapy. Nat Rev Clin Oncol. 2019;16:356–71.

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