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Intestinal metaplasia key molecules and *UPP1* activation via *Helicobacter pylori* /NF-kB: drivers of malignant progression in gastric cancer

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Abstract

Gastric cancer (GC) remains a significant global health challenge due to its high morbidity and mortality rates. The development of GC is a multi-hit process and the exploration of precancerous lesions is crucial. To elucidate the molecular and cellular dynamics underlying gastric carcinogenesis, we conducted an integrative single-cell RNA sequencing analysis of 26,028 high-quality cells from gastric antral mucosa biopsies across various stages, including non-atrophic gastritis, chronic atrophic gastritis, intestinal metaplasia, and early gastric cancer. By constructing a detailed single-cell atlas, we identified distinct epithelial cell subpopulations and their corresponding molecular signatures. We focused on the biological link between gastric epithelial cells and cancer cells. Notably, we observed that gland mucous cells acquired an intestinal-like stem cell phenotype during metaplasia, with *MUC6*, *MUC2* and *OLFM4* emerging as the specific markers for unique endocrine cells in early malignant lesions. Additionally, our analysis highlighted *UPP1* as a key oncogene, with its expression progressively increasing from normal epithelial cells to malignant cells. *UPP1* upregulation was shown to promote GC cell proliferation and migration, implicating it in the oncogenic process. Further, we explored the impact of *Helicobacter pylori* infection on gene expression, revealing that *Helicobacter pylori* infection upregulates *UPP1* via the NF-κB pathway. Our cell-cell communication analysis underscored the significant role of the Macrophage migration inhibitory factor pathway in the tumor microenvironment, contributing to GC progression. Various key molecules involved in intestinal metaplasia, along with *UPP1* and the Macrophage migration inhibitory factor pathway, collectively illustrate the multifaceted nature and complexity of gastric cancer evolution, highlighting the cumulative impacts that drive tumorigenesis.

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Introduction

Gastric cancer (GC) remains one of the most prevalent and deadly malignancies worldwide. Annually, it accounts for approximately large numbers of new cases and deaths [1]. Notably, the risk of developing GC increases with age and is approximately twice as high in men as in women. Major risk factors include *Helicobacter pylori* (*H.pylori*) infection, high-salt diet, smoking, alcohol consumption, obesity, and genetic predispositions. Despite advances in diagnosis and treatment, the prognosis for advanced GC remains poor, necessitating a deeper understanding of its pathogenesis and progression [2].

GC is a highly heterogeneous disease, and its molecular classification has become a critical aspect of understanding its pathogenesis and progression. The Cancer Genome Atlas (TCGA) has identified four major molecular subtypes of gastric cancer: Epstein-Barr virus (EBV/human herpesvirus 4 (HHV4))-positive, microsatellite instability, genomically stable, and chromosomal instability subtypes. Each subtype is characterized by distinct genomic and molecular alterations that influence tumor behavior, prognosis, and treatment response [3, 4]. With advancements in genomic technologies, we are increasingly able to uncover additional potential driver alterations involved in gastric cancer, which may lead to the identification of clinically significant biomarkers and potential therapeutic targets. Further refinement of gastric cancer research at the molecular level will greatly aid in selecting optimal treatment strategies for gastric cancer patients. Previous research has extensively explored the genetic, molecular, and environmental factors contributing to gastric carcinogenesis [5, 6]. These studies have identified numerous genes and pathways involved in GC, such as those related to cell proliferation, apoptosis, and DNA repair [7–9]. However, most studies have limitations, including insufficient resolution at the single-cell level and the inability to comprehensively map the dynamic changes from precancerous lesions to full-blown cancer. Our prior research has investigated the molecular mechanisms of GC, yet gaps remain in understanding the cellular heterogeneity and specific gene roles in the dynamic evolution from preneoplasia to GC.

We utilized single-cell RNA sequencing (scRNA-seq) with the GSE134520 dataset to analyze gene expression at a high resolution and identify distinct cell populations and their interactions within the tumor progression. Additionally, we conducted differential expression and enrichment analyses, pseudotime trajectory mapping, and cell communication studies to map the functional landscape of these genes in GC. Our research strategy included validating findings through PCR experiments and investigating regulatory mechanisms affecting gene expression.

In this study, we focus on the role of specific genes, including *MUC6*, *MUC2*, and *OLFM4*, during the intestinal metaplasia (IM) stage, as well as the uridine phosphorylase 1 (*UPP1*) gene. These genes play critical roles in the progression from precancerous lesions to GC. *UPP1* is known to play a critical role in tumor metabolism, particularly under conditions of nutrient deprivation. By breaking down uridine into ribose-1-phosphate, *UPP1* can channel this product into the glycolytic pathway, effectively providing an alternative energy source when glucose availability is limited. This mechanism allows tumor cells to maintain energy production and sustain growth under metabolic stress. Understanding their roles is crucial as they represent potential biomarkers and therapeutic targets for GC.

The results of our study provide a detailed characterization of gene roles in GC. We found that during the progression from IM to GC, *MUC6*, *MUC2*, and *OLFM4* play significant roles, along with *UPP1*. These findings highlight the importance of these genes in the early stages of gastric carcinogenesis and their potential as therapeutic targets. Our study offers new insights into the molecular and cellular dynamics of GC, contributing to future clinical applications and personalized treatment strategies.

Materials and methods

Patient enrollment and specimen collection

Specimens were collected from patients at the Affiliated Hospital of Yangzhou University. Basic clinicopathological features of the enrolled patients, including age, sex, *H.pylori* status and histopathological diagnosis (gastritis, atrophy, IM, and GC). Ethical approval for the study was obtained from the institutional review board of the hospital. The inclusion criteria were as follows: (1) diagnosis confirmed by histopathological examination; (2) no previous history of other malignant tumors; (3) no prior chemotherapy or radiotherapy; and (4) patients provided written informed consent. Each specimen was collected during routine endoscopic or surgical procedures, ensuring a representative sample from each patient's gastric tissue. GCs were classified as *H.pylori*-negative based on the following criteria: (1) no antibiotic eradication history; (2) no atrophic appearance by endoscopy; (3) no atrophic appearance by histology; (4) negativity in two or more tests such as rapid urease, urease breath, serum antibody, stool antigen and serum pepsinogen tests. All gastric tissue specimens were classified based on

pathological diagnosis and further divided into *H.pylori* infection groups (positive or negative).

ScRNA-seq sequencing data collection

We conducted a comprehensive search of public databases to identify relevant single-cell RNA sequencing datasets for our study on the dynamic evolution of GC. Four datasets were selected based on their relevance and data availability: GSE134520. The datasets from GSE134520 was obtained from the Gene Expression Omnibus (GEO) repository [10].

ScRNA-seq sequencing data processing

We used the GSE134520 dataset for our single-cell RNA sequencing analysis, which comprises 13 samples categorized into four groups: normal adjacent gastric tissue (NAG, $N=3$), chronic atrophic gastritis (CAG, $N=3$), intestinal metaplasia (IM, $N=6$), and early gastric cancer (EGC, $N=1$). The cell matrix file for each sample was imported into R using the CreateSeuratObject function from the Seurat V5 [11]. Cells with adequate gene detection and transcript counts were selected for further analysis. Additionally, cells with high mitochondrial gene transcripts were considered potentially apoptotic and were excluded. Doublets were identified and removed using DoubletFinder with default settings for each sample. To minimize noise and expression artifacts, specific genes associated with mitochondria, heat-shock proteins, ribosomes, and dissociation were excluded. After filtering out low-quality cells, we conducted integration and batch-effect correction within each single-cell dataset using Harmony. Subsequently, all samples were further integrated, and batch effects were adjusted again using Harmony [12]. This rigorous data processing resulted in a high-quality single-cell dataset, which was then used for downstream analysis.

Clustering and cell type annotation

The integrated single-cell RNA sequencing data were scaled, followed by dimensionality reduction using principal component analysis. Clustering analysis was performed using the Louvain algorithm implemented in the Seurat package, applied to the principal component analysis-reduced data with a specified number of principal components (PCs) to be determined. The resolution parameter was adjusted to achieve optimal clustering results. For visualization, uniform manifold approximation and projection (UMAP) was employed using the top PCs to display cell clustering. UMAP clusters cells based on their transcriptomic features, capturing similarities in gene expression profiles. Differentially expressed genes (DEGs) for each cluster were identified using the Wilcoxon test, considering only genes with an adjusted Pvalue <0.05 and a logFC >0.25 as marker genes. Clusters were annotated based on these marker genes specific to cell types and classic cell marker genes (Table S2). Subclustering for each major cell type was performed by extracting data for each cell type from the integrated dataset, followed by clustering with cell type-specific resolution as described above.

Prediction of cell trajectory

Trajectory analysis was conducted using Monocle2 to characterize the progression from normal gastric cells to tumor cells and to determine lineage differentiation among these cells. We imported the integrated gene expression data into Monocle2 and ordered the cells based on DEGs between subclusters, selecting significant genes with a q-value <0.01. The differentiation trajectory of gastric cells was inferred following dimension reduction using the DDRTree method and cell ordering with Monocle2's default parameters [13].

Cell–cell communication analysis

Cell-cell communication analysis was performed to evaluate the differences in putative communication modules between cell types across various stages of GC progression using the CellChat pipeline [14]. We integrated gene expression data and utilized the default CellChatDB ligand-receptor database (Figure S1) to infer cell type-specific communication by identifying overexpressed ligands or receptors within each cell group, subsequently determining enhanced ligand-receptor interactions. Additionally, NicheNet analysis was conducted to investigate the signaling mediators involved in ligand-receptor pairing among cancer cells, immune cells, endothelial cells, and fibroblasts. For both sender and receiver groups, genes from the signaling pathway of interest, expressed in at least 10% of the cells, were included in downstream analysis. The NicheNet analysis followed established protocols to rank potential ligands, infer receptors, and identify top-predicted target genes of ligands, providing a comprehensive understanding of cell-cell communication in GC.

Bulk transcriptomic data collection and processing

Bulk RNA sequencing data from three datasets—GSE118916, GSE103236, GSE2685, GSE78523, GSE70394, GSE108305, GSE5081 and TCGA-STAD—were collected and processed for analysis. Data acquisition involved downloading raw count data from the GEO and The Cancer Genome Atlas (TCGA) databases. For GEO datasets GSE118916, GSE103236, GSE2685, GSE78523, GSE70394, GSE108305 and GSE5081, raw counts were normalized using the DESeq2 package in R. For TCGA-STAD, RNA-seq data were obtained from the Genomic Data Commons portal and processed similarly. Subsequent analysis included differential gene expression

analysis to identify key genes associated with GC progression. Visualizations such as volcano plots, and survival curves were generated using R packages like ggplot2 to present the results.

Cell culture

GC cell lines AGS and HGC27, as well as the normal gastric cell line GES-1, were sourced from the platform at Yangzhou University Affiliated Hospital. These cell lines underwent short tandem repeat profiling for authentication and mycoplasma testing to ensure contamination-free cultures. Cells were maintained in RPMI-1640 medium (AGS and HGC27) or DMEM (GES-1) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere containing 5% CO2. Regular monitoring and routine subculturing were performed to maintain optimal growth and viability for experimental procedures. AGS: AGS cells are derived from a patient with gastric adenocarcinoma. They are moderately differentiated and commonly used to study gastric cancer biology, including *H. pylori* infection and its effects on gastric epithelial cells. AGS cells are characterized by microsatellite stability (MSS) and are TP53 mutant, which makes them a relevant model for studying p53-related pathways in GC; HGC27: HGC27 is derived from a poorly differentiated human gastric carcinoma. Unlike AGS, HGC27 cells are microsatellite instability-low and are wild-type for TP53. This difference provides a contrast in molecular behavior and is valuable in studying tumor progression and invasion mechanisms in GC.

H.pylori culture

H.pylori strain ATCC 43,504 was utilized for the study. The bacteria were cultured on Columbia agar plates supplemented with 7% defibrinated sheep blood and incubated under microaerophilic conditions (10% CO_2, 5% O_2, 85% N_2) at 37 °C for 2 days. For liquid cultures, *H.pylori* was grown in Brucella broth supplemented with 10% fetal bovine serum (FBS) under the same microaerophilic conditions. Bacterial growth was monitored by measuring optical density at 600 nm (OD600). The cultures were maintained and subcultured every 48 h to ensure the bacteria remained in the logarithmic growth phase for subsequent experiments.

Cell infection and transfection

Cell infection and transfection were conducted using siRNAs specific to the target genes, with sequences detailed in the supplementary materials (**Table S3**). Control and target siRNAs (Proteinbio, Nanjing, China) were transfected into AGS and HGC27 GC cells using Lipofectamine 3000 (Invitrogen, USA) following the manufacturer's protocol. Successful gene interference was confirmed by RT-qPCR analyses, demonstrating significant reduction in gene expression levels.

Quantitative real-time PCR (RT-qPCR)

Quantitative real-time PCR (RT-qPCR) was utilized to quantify gene expression in gastric epithelial and cancer cells. Gastric cells, cultured under standard conditions and treated with *H.pylori* as necessary, underwent RNA extraction using TRIzol, followed by purity and concentration assessment via NanoDrop. The RNA was then reverse-transcribed using the HiScript III All-in-One RT SuperMix Perfect for qPCR (R333-01, Vazyme, China) and RT-qPCR was performed using the SYBR Green PCR Master Mix. Specific primers for target genes and reference genes were shown in the Supplementary Material (Table S4). Thermal cycling included initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min, with melt curve analysis to ensure specificity. Relative gene expression was calculated using the 2^(-ΔΔCt) method, with data presented as mean ± SD from triplicate experiments, and statistical significance determined by Student's t-test ($p < 0.05$).

Cell proliferation and migration assays

Cell proliferation was assessed using a colony formation assay, while cell migration was evaluated using a transwell migration assay, both targeting the *UPP1* gene in GC cell lines AGS and HGC27. For the colony formation assay, cells were seeded in 6-well plates at a density of 500 cells per well and cultured for 14 days. Colonies were fixed with 4% paraformaldehyde, stained with crystal violet, and counted using ImageJ software. For the transwell migration assay, cells were seeded into the upper chamber of transwell inserts with an 8 μm pore size, in serum-free medium. The lower chamber contained medium supplemented with 10% FBS as a chemoattractant. After 24 h of incubation, cells that had migrated to the lower surface of the membrane were fixed, stained, and counted using ImageJ software.

Online database

The gene expression levels were analyzed in Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia2.cancer-pku.cn/#index). GEPIA provides access to RNA sequencing expression data from TCGA and GTEx, allowing us to compare gene expression in tumor versus normal tissues. The Human Protein Atlas (https://www.proteinatlas.org/) offered immunohistochemical images of four genes, highlighting their expression levels in gastric cancer tissue compared to adjacent non-cancerous tissues. Kaplan-Meier Plotter (https://kmplot.com/analysis/index.php?p=background) was used to query the survival prognosis of target genes in GC.

Statistical analysis

R software (version 4.3.1) and GraphPad Prism (version 9) were used. Each in vitro experiment was repeated three times. Quantitative variables were analyzed using a Student t-test for Gaussian distribution and non-parametric tests for non-Gaussian distribution. For single-cell RNA-sequencing data, the AddModuleScore function was used to score pathway activity, and gene expression differences between clusters were tested using the Wilcoxon rank-sum test. Differences were considered statistically significant at $p<0.05$ ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

Results

A single-cell expression atlas of GC ecosystems during tumor progression

To comprehensively explore the dynamic changes in GC, we integrated scRNA-seq on a cohort of samples representing various stages of GC progression. This analysis included a total of 13 specimens (Fig. 1A), comprising 3 NAG, 3 CAG, 6 IM, and 1 EGC. All sample was paired to ensure comprehensive coverage of the GC progression cascade, with detailed pathological and clinical information available in Supplementary Data (Table S1). Following stringent quality control and data filtration processes, we retained 26,028 single cells for further analysis. We employed the Harmony integration algorithm to mitigate batch effects, followed by graph-based clustering and marker-based annotation to classify the cells (Fig. 1B). We compared the gene expression patterns of each cell type and using heat map shows the top five genes (Fig. 1C).

We defined the major cell types by well-established canonical marker genes [15]. This resulted in the identification of eight major cell clusters, including epithelial cells ($n=17,139$), cancer cells ($n=787$), mast cells ($n=330$), Enterocyte ($n=2,365$), fibroblasts ($n=1,386$), B cells ($n=2,208$), T cells (SMCs) ($n=1,550$), and macrophage ($n=263$). Epithelial cells were identified based on the expression of *MUC5AC* and *MUC6*, mast cells were characterized by *TPSAB1* and *TPSB2*, Enterocyte by *FABP1*, *CA1*and *VIL1*, fibroblasts by *DCN* and *PDPN*, B cells by *CD19* and *CD79A*, T cells by *CD2* and *CD3D*, macrophage by *CD14* and *CD163* (Fig. 1D). The cell composition and infiltration fractions across different pathological stages of GC revealed significant heterogeneity. The distribution, number, and percentage of each cell cluster varied markedly between the stages, indicating dynamic changes in the ecosystem during GC progression (Fig. 1E).

Molecular features of the single-cell expression profiles for epithelial cells across different lesions

To further investigate the dynamic evolution of GC, we focused on the epithelial cell subpopulations. Epithelial cells were extracted and further divided into distinct subgroups, including gastric mucous cells (GMC), pyloric mucous cells, chief cell, etc. based on specific marker genes and clustering analysis (Fig. 2A) [16, 17]. Gastrointestinal mucus is the first line of defense against bacteria. There are a large number of disease biomarkers expressed in the GMC cell population, so we chose the GMC cell population for further exploration. IM is an important physiological stage in the dynamic evolution of GC. Our combined analysis of the GMC cell population and the external dataset GSE78523 showed high expression of *OLFM4* and *MUC2* and low expression of *MUC6* in IM tissues (Fig. 2B). According to the current research, *MUC6* and *MUC2* are the classic markers of IM and carcinogenesis characterized by elevated production of *MUC2* [18, 19], as well as a reduction in the production of the protective mucin *MUC6* [20, 21]. In addition to this, we found that *OLFM4* was elevated when IM occurred in gastric tissue, which recognized as a classical marker for intestinal metaplasia (IM) in gastric tissues. Several studies have established *OLFM4*'s role in the context of gastric carcinogenesis. For instance, elevated levels of *OLFM4* are often indicative of ongoing metaplastic changes, serving as a signal of the gastric epithelium's response to injury and inflammation. At the same time, we found that the different stages of lesions of GMC different pathways, including the CAG inflammation and tumorigenesis of subsequent lesions (Fig. 2C). The expression of *MUC2* and *OLFM4* showed an increasing trend during the dynamic evolution of epithelial cells into tumor cells, while that of *MUC6* was the opposite (Fig. 2D). With this dynamic expression pattern, we speculate that these genes play a key role in driving the physiological changes. As shown in Fig. 2E, in GMC cells, the proportion expressing *MUC2* and *OLFM4* increased while *MUC6* decreased as epithelial cells underwent atrophy and IM and tumorigenesis, and the expression of the two groups of genes showed a negative correlation. The results also reflect the physiological changes in the process is the product of the common genetic interactions, rather than the result of a single gene regulation [22]. These results reflect the epithelial cells in many stages in the development of self-renewal and phenotypic changes, this may be the key factor for tumor progression.

Integrative cancer cell subgroup data analysis identifying *UPP1*^{high}cancer cells and the association of *UPP1* with patient prognosis

We further performed dimensionality reduction clustering of cancer cell populations, and the results revealed four cancer cell subsets, including *UPP1*^{high} cancer cells, *RNF43*^{high} cancer cells, *STRA6*^{high} cancer cells and *TAOK1*^{high} cancer cells (Fig. 3A). In this study, we used GEPIA and Kaplan-Meier Plotter databases to analyze the

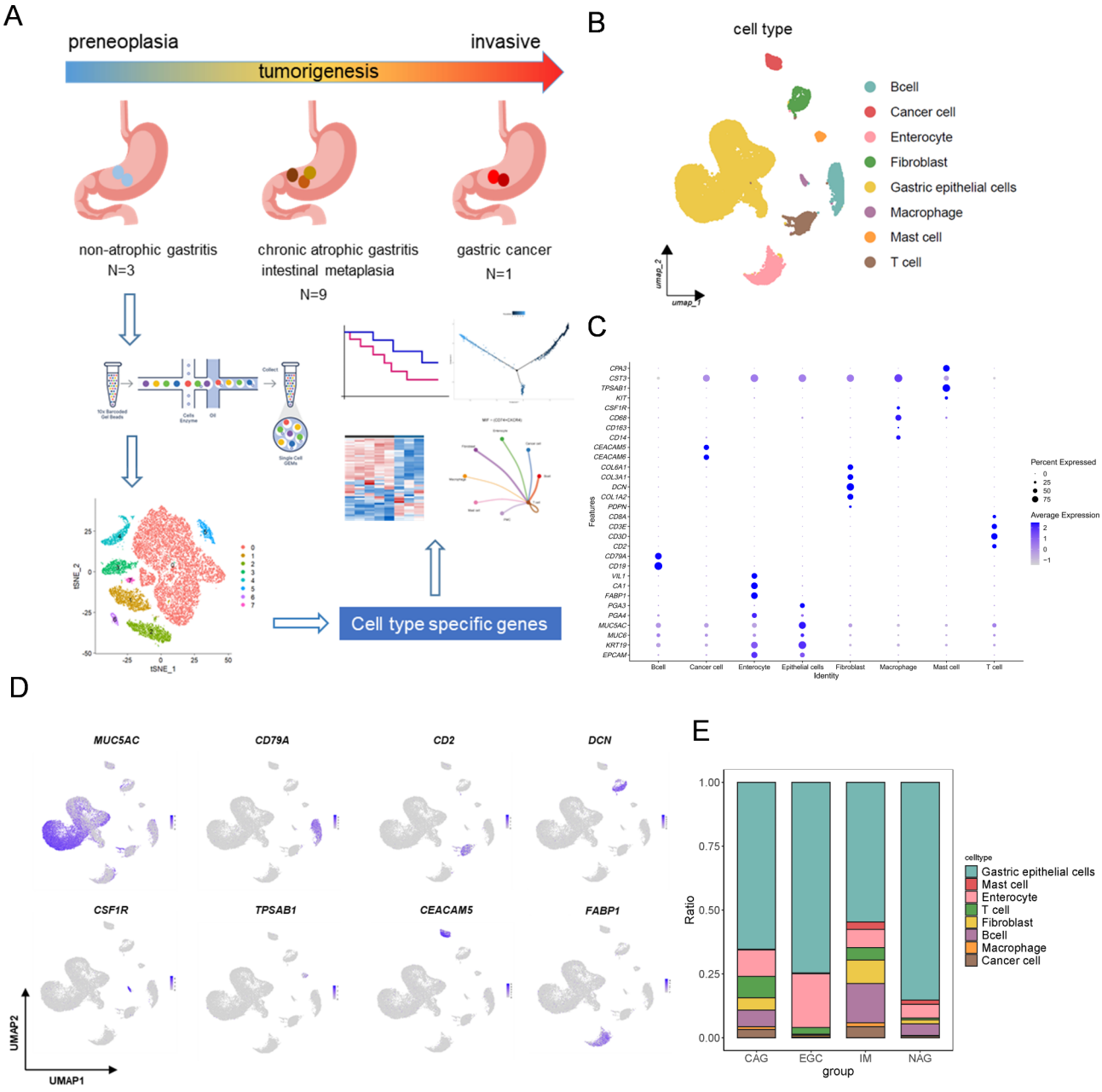

Fig. 1 Single-cell expression profiling of human premalignant and GC. (**A**) A schematic graph shows the study design. (**B**) Uniform manifold approximation and projection (UMAP) visualization of 26,028 cells from 13 samples, showing 8 clusters in different colors. (**C**) Cell-type markers. The relative expression level of genes across cells is shown, sorted by cell type. (**D**) UMAP of scRNA-seq showing eight major cell types identified by marker gene expression (MUC5AC: Epithelial cells; CD79A: B cells; CD2: T cells; DCN: fibroblasts; CSF1R: Macrophage; TPSAB1: mast cells; CEACAM5: cancer cells; FABP1: Enterocyte). (**E**) Proportion of 4 major pathology types showed in bar plots in different sample types

expression levels of *UPP1*, *RNF43*, *STRA6*, and *TAOK1* in gastric cancer tissues (Figure S2). Immunohistochemical results shows the four representative genes in GC tissues are all high expression (Fig. 3B), indicating the biological representativeness of the cell subsets we identified. At the same time, we visually displayed the representative genes of four groups of cancer cells using heat maps (Fig. 3C). In different cell types, we can observe a clear segregation of the expression of four genes in tumor cells. Interestingly, *RNF43* and *TAOK1* were also highly expressed in fibroblasts, suggesting their involvement in the regulation of tumor cells by tumor-associated fibroblasts [23] (Fig. 3D). In our study, we further validated the expression of key genes in gastric cancer by introducing external datasets GSE118916, GSE103236, and GSE2685. Through this external validation, we demonstrated consistency

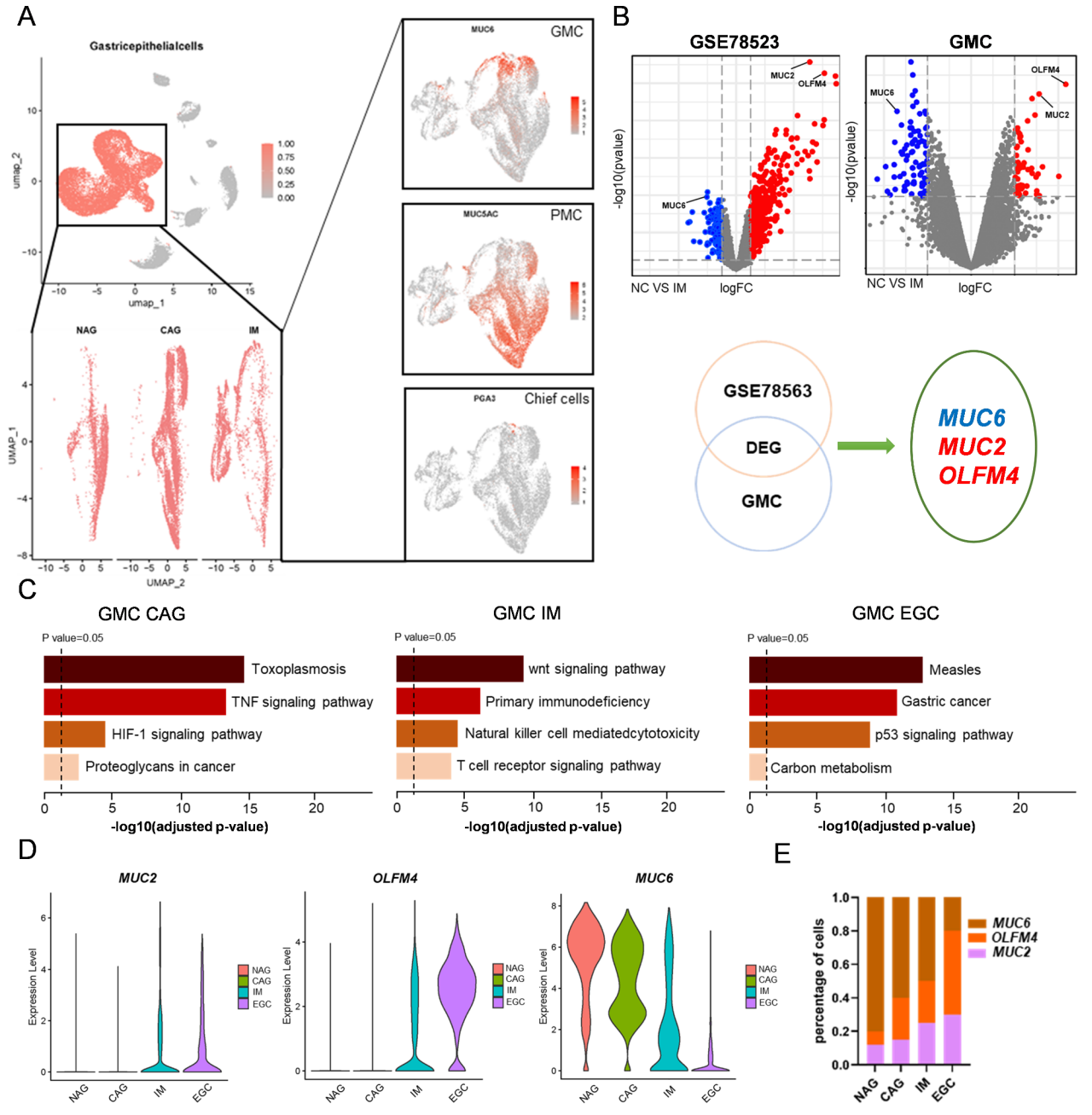

Fig. 2 The scRNA profiles for gastric epithelial cells across different Lesions and key genes. (**A**) The UMAP plot showing the major cell populations (GMC, PMC, chief cells). (**B**) Volcano plot from GSE78523 and GMC cell population highlighting the three DEGs, MUC6, OLFM4 and MUC2, in intestinal metaplasia during the dynamic evolution from preneoplasia to gastric cancer. (**C**) The most enriched pathways for upregulated genes for GMCs from samples in CAG, IM, and EGC lesion, respectively. (**D**) Violin plots display the distribution of expression of MUC2, OLFM4, and MUC6 across multiple lesions. (**E**) Bar chart showing the relative percentage of cells expressing MUC6, OLFM4 or MUC2 alone across different lesions

in gene expression across different cohorts, strengthening the reliability of our conclusions about the role of these key genes in gastric cancer progression (Figure S3). In addition, we collected normal gastric tissues and gastric cancer tissues to further validate *UPP1* expression levels. The results were consistent with our previous analysis, reinforcing the conclusions of our study. In bulk-RNA-seq data from TCGA-STAD cohort, $UPP1^{high}$ cancer cells abundance was significantly associated with overall survival (OS), indicating that $UPP1^{high}$ cluster has an aggressive phenotype and contributes to GC progression (Fig. 3E). Furthermore, TCGA data showed the association with *UPP1* expression and clinicopathologic variables. As shown in Fig. 3F, increased expression of

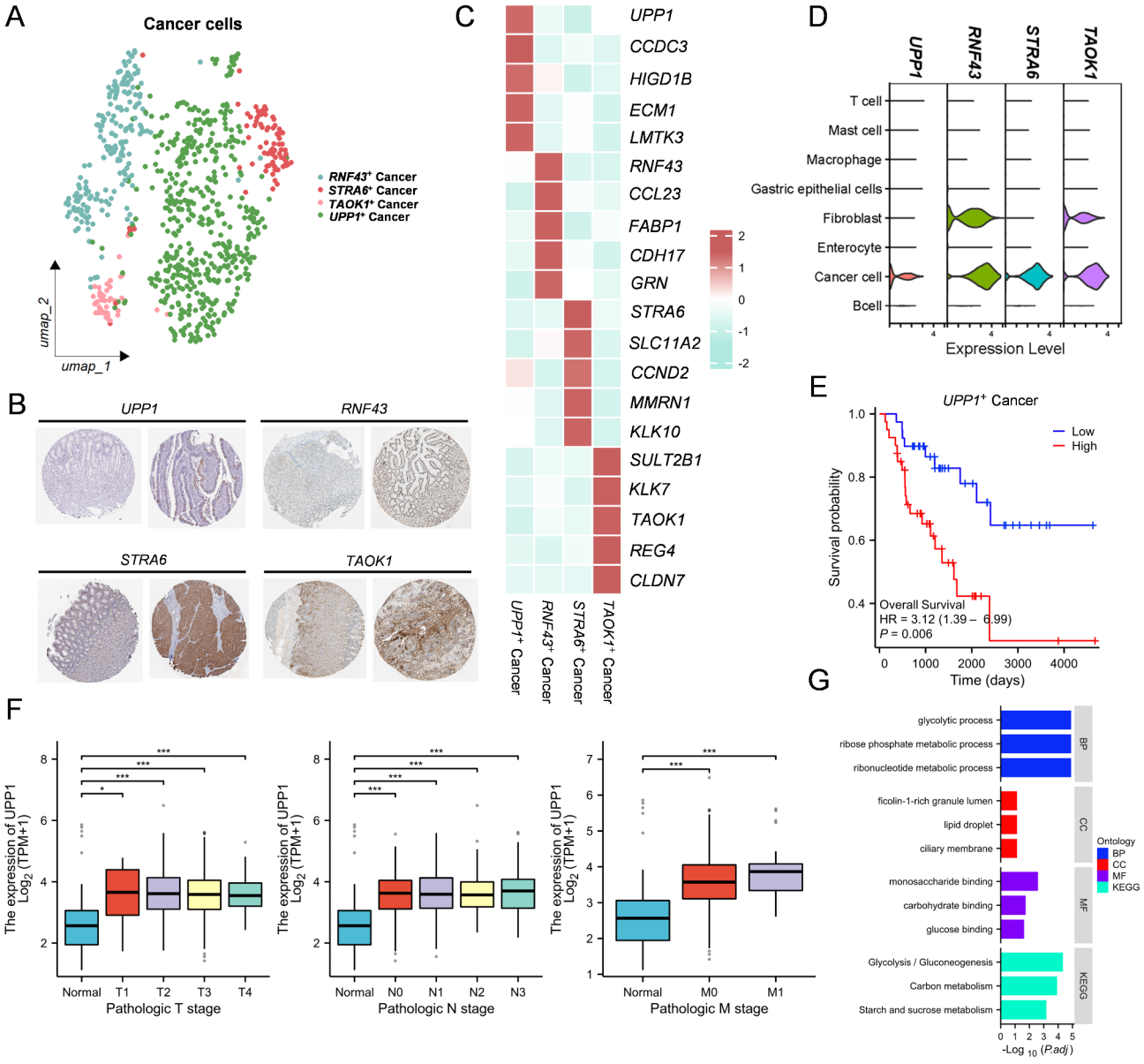

Fig. 3 Integration of cancer cells and identification of prognostic-related cancer cell populations. (**A**) The UMAP plot displaying tumor cell clusters. 4 distinct tumor cell clusters were identified. The top marker gene for each of these clusters is presented. (**B**) Immunohistochemistry of marker genes in cancer and adjacent tissues. (**C**) The heatmap showing the mean expression of the top five marker genes for the 4 cancer cell clusters. (**D**) Boxplot for the distribution of expression of the cancer marker UPP1, RNF43, STRA6 andTAOK1 in diverse cell types. (**E**) The Kaplan-Meier curves showed UPP1^{high} cancer cell are associated with worse overall survival (OS) in TCGA-STAD cohort. (**F**) Association of UPP1 mRNA expression with TNM stage. T0: No evidence of primary tumor; T1-T4: Size and/or extent of the primary tumor; N0: no lymph node metastasis; N1: nearby lymph node metastasis; N2: distant lymph node metastasis; M0: no distant metastasis; M1: distant metastasis. (**G**) GO|KEGG analysis based on UPP1-related genes. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

UPP1 correlated significantly with the TNM clinical stage grade. To further determine the biological characteristics of *UPP1*, KEGG enrichment analysis was performed on the up-regulated genes of *UPP1*. The results showed significant enrichment in pathways related to glucose metabolism (Fig. 3G). It has been reported that *UPP1* can regulate the occurrence and development of tumors as a key regulatory enzyme in glycolysis. *UPP1* plays a crucial role in pyrimidine metabolism, specifically in the salvage pathway of uridine metabolism. *UPP1* catalyzes the conversion of uridine into uracil and ribose-1-phosphate, a key metabolite that can enter the pentose phosphate pathway or be further utilized in downstream glycolytic pathways. In this context, ribose-1-phosphate generated by *UPP1* provides an alternative energy source to glucose, fueling glycolysis and supporting the heightened metabolic demands of rapidly proliferating cancer cells. This process is particularly important in tumor

environments where glucose availability may be limited, allowing cancer cells to sustain their energy production and biosynthesis requirements through alternative metabolic routes [24, 25]. While there is limited research specifically on *UPP1* in gastric cancer, its role in other cancer types suggests that it may be a key player in facilitating tumor growth through altered uridine metabolism. Therefore, we focused specifically on the *UPP1*^{high} cancer cell population and the role of *UPP1* in the dynamic changes of GC.

H.pylori is a high risk factor for the dynamic evolution from precancerous to GC

H.pylori infection is strongly associated with GC development. *H.pylori* infection leads to IM and dysplasia of the gastric mucosa, which are the prodromal state of GC [26]. IM and hyperplasia of cells undergo malignant transformation in the continuous inflammation environment easily [27, 28]. To analyze the influence of *H.pylori* infection on the dynamic changes in GC, we categorized the GC cells into *H.pylori* -positive and *H.pylori* -negative groups (Fig. 4A). Pathway enrichment of genes upregulated by *H.pylori* infection showed that these genes were involved in inflammatory and tumor-related biological processes, including NF-κB pathways (Fig. 4B). *H.pylori* as long-term presence of inflammation in the gastric environment factors, and the key of biological gene together to promote the transformation of precancerous lesions [29, 30]. We found that *UPP1* expression is elevated after *H.pylori* infection of gastric tissue in tumor cell populations. In gastric epithelial cells, when infected with *H.pylori*, the expression of *MUC6* will decline, at the same time the expression of *OLFM4* will rise (Fig. 4C, **D**, **E**). In the external validation, *UPP1* was highly expressed in AGS cells or GC tissue after *H.pylori* infection, and this result was validated at the RNA level (Fig. 4F, Figure S4). *UPP1* as a uridine metabolizing enzyme has attracted the attention of researchers recently [31]. It has been shown that *UPP1* expression is regulated by the NF-κB pathway [32–34]. Therefore, we hypothesized that *H.pylori* could activate the NF-κB pathway through inflammatory response, leading to the binding of P65 to the promoter of *UPP1*, thereby promoting the expression of *UPP1*, and thus affecting the occurrence and development of GC. We utilized the AGS cell line to investigate the regulatory effect of *H.pylori* on *UPP1* expression and the experiments were performed under conditions of H. pylori infection with a multiplicity of infection (MOI) of 1:100. We also collected GC tissues and *H.pylori*-infected tissues, and validated *UPP1* expression levels through PCR analysis. The results from this experiment further confirmed the conclusions drawn from our data analysis (Figure S6). When we knocked down *P65*, the expression of *UPP1* was significantly inhibited, which indicated the regulatory effect of *H.pylori* on *UPP1*, which was consistent with our conjecture and the published results (Fig. 4G, H, I). AlphaFold3 as emerging biological model, play an important role in the field of molecular interactions [35]. We used the AlphaFold3 tool to perform a three-dimensional model reduction of *UPP1* regulated by *P65*, which vividly showed the mode of molecular regulation (Fig. 4J). The gene sequences used by AlphaFold3 are all presented in the Supplementary material (Supplementary data Data1). Combined with previous studies and our experimental verification, the *H.pylori* -NF-κB-*UPP1* axis is formed, which provides a new idea for *H.pylori* to promote gastric carcinogenesis.

UPP1 plays an important role in promoting GC occurrence and development

Trajectory analysis was performed to decipher the cell trajectory of GC from normal epithelial cells to cancer cells to reveal the origin of GC at the single-cell level and we can clearly find that the expression of *UPP1* is continuously increased in the process of carcinogenesis (Fig. 5A, **B**). The dynamic expression changes of *UPP1* again suggested its oncogenic role in tumorigenesis. We collected tissues from different patients in our clinical practice, including all pathological stages of GC lesions, including gastritis, atrophy, IM, and GC. Figure 5 C illustrates the endoscopic progression from gastritis, atrophy, intestinal metaplasia (IM), to gastric cancer, mirroring the molecular shifts discussed in this study. *UPP1*'s upregulation, as demonstrated in our findings, aligns with the transition from these pre-malignant stages to the malignant state, highlighting its potential role in promoting gastric cancer development. The expression of *UPP1* was gradually increased in different stages of gastric tissue, which was consistent with the previous trajectory analysis. To further elucidate the functional significance of *UPP1*, we conducted proliferation and migration assays. These experiments demonstrated that the knockdown of *UPP1* expression significantly decreased the proliferative and migratory capacities of GC cells, confirming its oncogenic role (Fig. 5D, E). As a uridine phosphorylase, *UPP1* plays a crucial role in the uridine salvage pathway, which is pivotal for nucleotide metabolism and cellular proliferation [34, 36] (Fig. 5F). We also explored the regulatory impact of *UPP1* on the uridine salvage pathway and its consequent effects on GC cell proliferation. Our findings indicate that *UPP1* modulates the uridine salvage pathway, thereby influencing the proliferation of GC cells (Fig. 5G). This regulatory mechanism highlighted the interplay between *UPP1* and cancer cell growth.

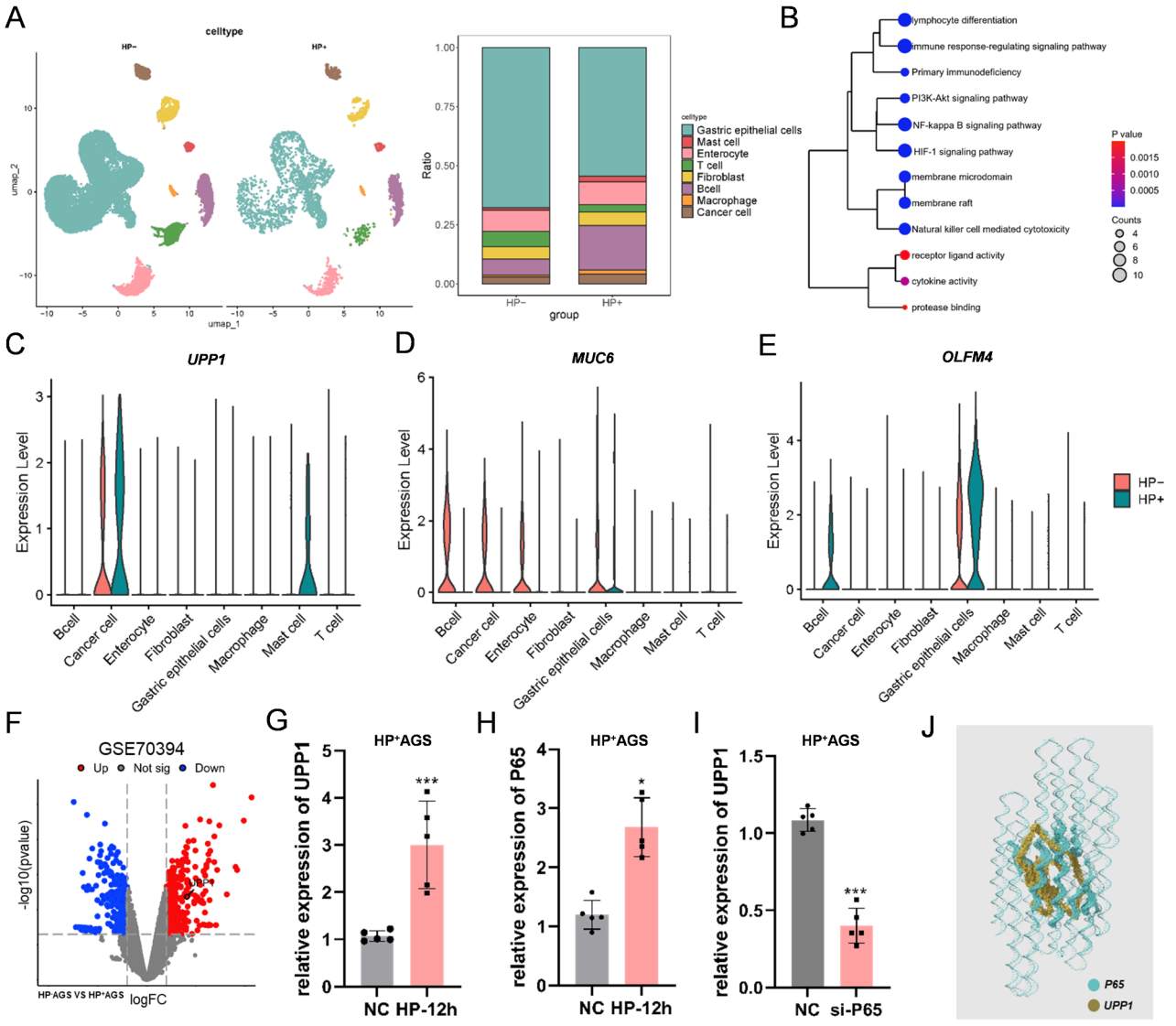

Fig. 4 Important role of Helicobacter pylori and key genes in tumorigenesis. (**A**) The UMAP plot and proportion of cells from *H. pylori*-infected samples and uninfected samples. (**B**) The most enriched pathways for upregulated genes for *H. pylori*-infected. (**C**-**E**) Violin plots display the distribution of expression of UPP1, MUC6 and OLFM4 across diverse cell types. (**F**) Volcano plot from GSE70394 highlighting the High expression level of UPP1 in *H. pylori*-infected AGS. (**G**, **H**) The expression levels of UPP1 and P65 were increased when *H. pylori* was infected. UPP1 expression levels drop when knock down P65. (**J**) AlphaFold 3 was used to predict the interaction between P65 and UPP1 promoters. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

MIF pathway is continuously activated from precancerous to GC

Communication and signaling between cells are essential for tumorigenesis [37]. The interacting pathways of NAG, CAG, IM and EGC stages were analyzed by CellChat (Fig. 6A). Macrophage migration inhibitory factor (MIF) is a classical pro-inflammatory cytokine that plays an important role in the link between inflammation and cancer [38] (Fig. 6B). In recent years, numerous studies have demonstrated that the expression level of MIF is significantly increased in a variety of tumor tissues and MIF promotes the occurrence and development of tumors [39–41]. We employed the AddModuleScore method to assess the activity of the MIF signaling pathway within the cancer cell population. Notably, we found that the cells characterized by high *UPP1* expression exhibited the highest scores for the MIF pathway. This observation indicates a strong correlation between *UPP1* and MIF, suggesting that *UPP1* upregulation is associated with increased MIF signaling in the tumor microenvironment (Figure S5). Meanwhile, we analyzed the receptor-ligand contribution of MIF pathway (Fig. 6C). This analysis aimed to uncover the role of MIF signaling in the dynamic evolution of GC and its impact across various cell populations. Interestingly, MIF pathway was rarely involved in cell-to-cell signaling in NAG, but was

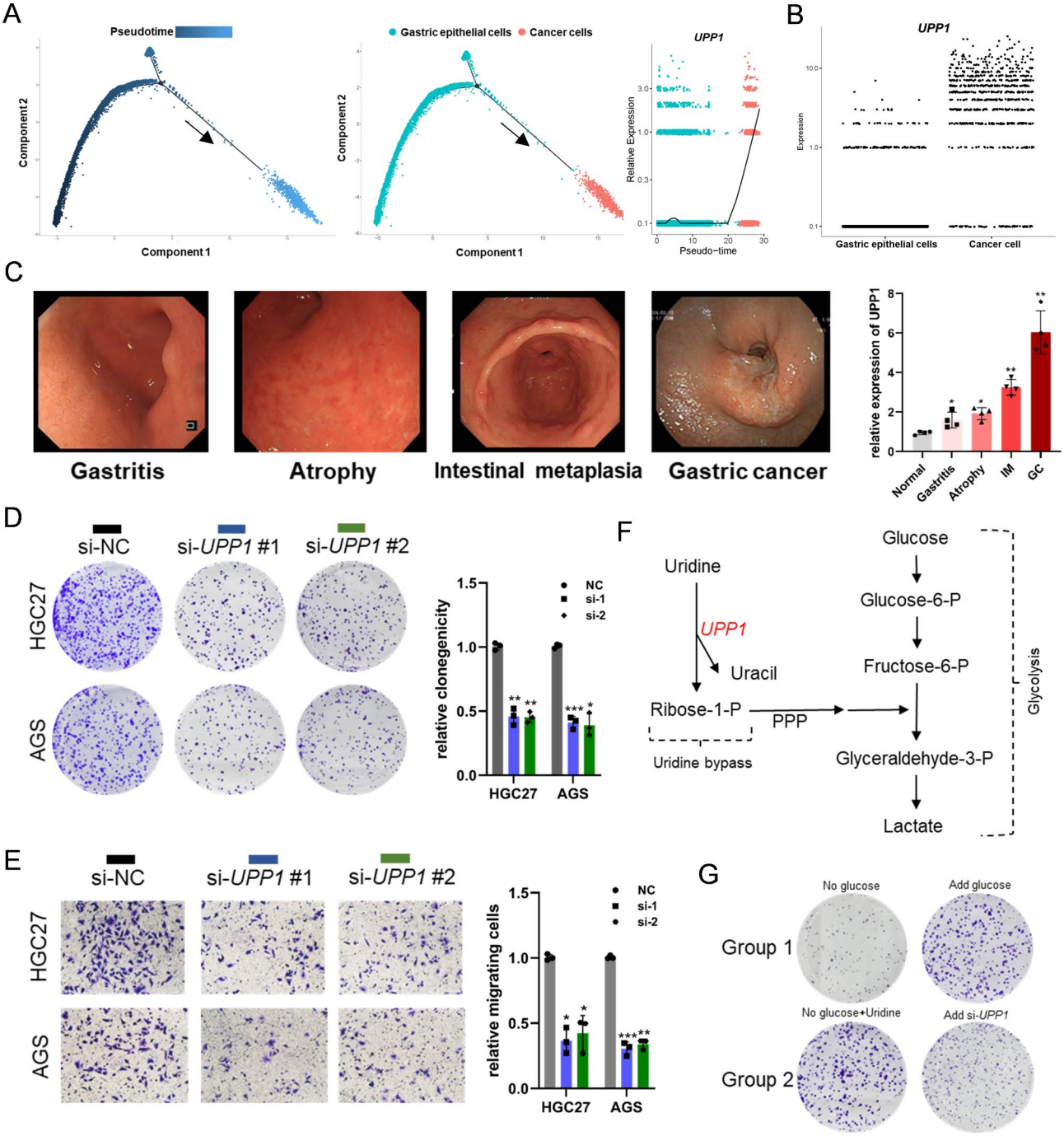

Fig. 5 UPP1 regulate the proliferation and migration of gastric cancer through uridine bypass. (**A**) Potential trajectory of epithelial cells to cancer cells inferred by Monocle2 and the expression of UPP1 increased gradually. (**B**) During the dynamic changes, the expression of UPP1 in tumor cells was significantly higher than that in normal gastric epithelial cells. (**C**) The expression of UPP1 was gradually increased in human tissues of gastritis, atrophy, intestinal metaplasia and gastric cancer. (**D**) cell proliferation was assessed by clone formation assay. (**E**) transwell assay and wound healing migration assay were employed to detect the migration ability of UPP1 knockdown cells. (**F**) Schematic of uridine-derived ribose catabolism integrating gene essentiality results in glucose versus uridine. (**G**) Colony-forming assays assessed GC cell proliferation in the presence of si-UPP1, glucose, uridine. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

significantly increased in CAG, IM and EGC (Fig. 6D). Increasing involvement of MIF pathway was observed in fibroblasts, reflecting its pro-tumor role in the tumor microenvironment. The high expression of MIF pathway in tumors also indicates that it is a key protein connecting the inflammation-tumor axis (Fig. 6E). The difference of MIF effect is probably the receptor and the ligand caused by the differences of expression in different cells.

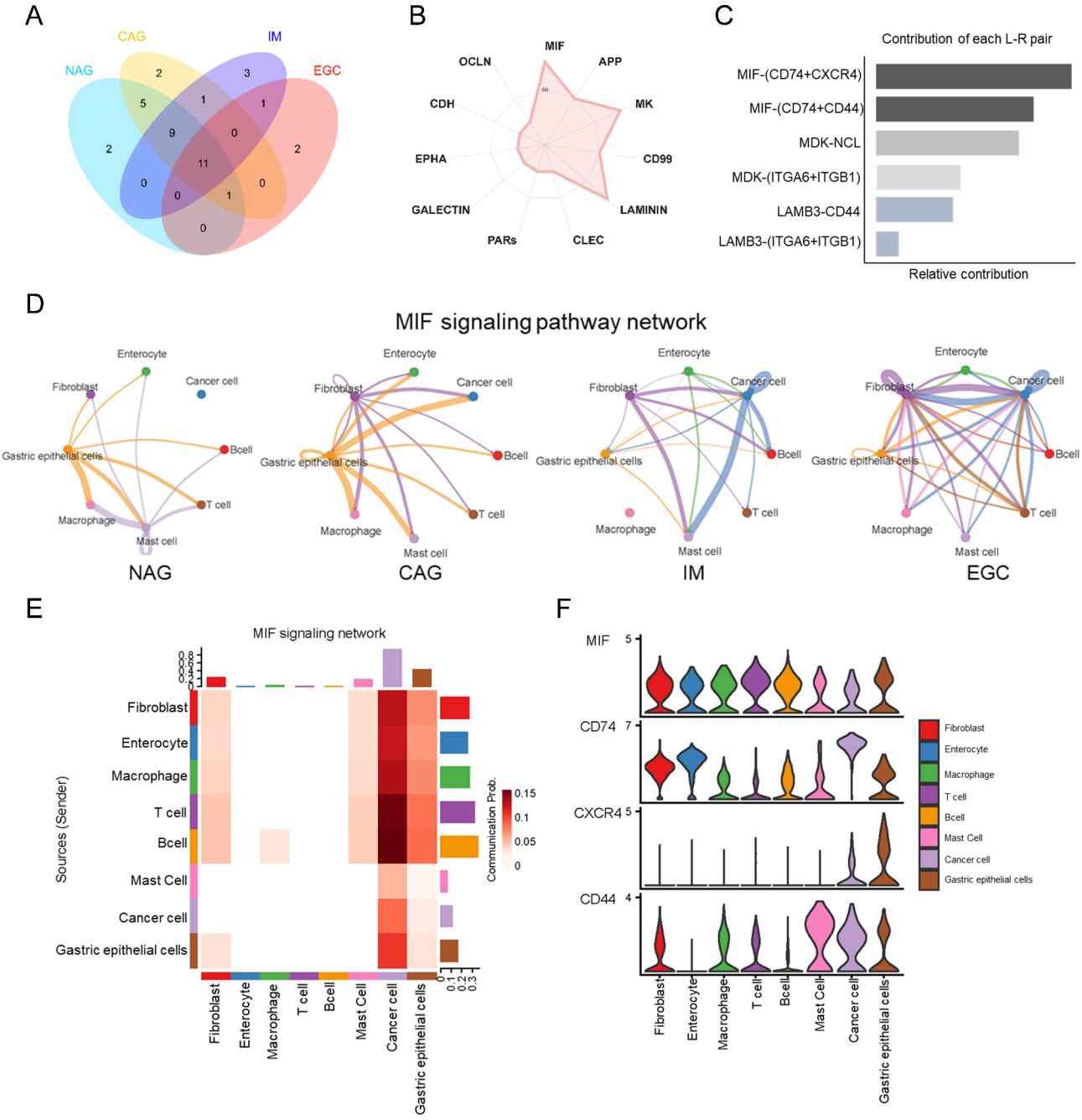

Fig. 6 Maps showing the role of the MIF signaling pathway in the dialog between cells. (**A**) Venn diagram shows the pathways involved in cells across multiple lesions. (**B**) Radar chart from CellChat data showing the major signaling pathways that mediate cell-to-cell interactions. (**C**) Contribution of receptor and the ligand of MIF pathways. (**D**) MIF signaling pathway interactions between eight specific cell types from scRNA-seq data. The maps show MIF pathway interactions cells in NAG, CAG, IM, EGC and IAC. (**E**) The communication strength of the MIF pathway across different cell types. (**F**) The expression patterns of MIF and its associated receptors in diverse cell types. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

The analysis delineated the expression patterns of MIF and its associated receptors, such as *CD74* and *CXCR4*, across different cell populations from precancerous to GC (Fig. 6F). Notably, MIF expression was elevated in specific cancer cell clusters, suggesting its involvement in promoting a pro-tumorigenic milieu. The differential expression of MIF receptors further highlighted the complexity of its signaling network, with various cell types exhibiting distinct receptor profiles, thereby modulating their response to MIF. In summary, the cell-cell communication analysis highlights the critical influence of the MIF pathway in the dynamic evolution from preneoplasia to GC. By demonstrating the pervasive impact of MIF across diverse cell populations and its role in

fostering a tumor-supportive environment, our findings pave the way for novel intervention strategies targeting this key signaling axis.

Discussion

GC remains a significant global health challenge, with high morbidity and mortality rates. Due to mild symptoms of early disease and low routine screening rates, most patients are diagnosed at advanced stages [42]. Despite advances in early detection and treatment, the molecular mechanisms driving the progression from precancerous lesions to GC are not fully understood. Previous studies have provided insights into the genetic and epigenetic changes associated with gastric carcinogenesis, yet a comprehensive understanding of the cellular and molecular dynamics involved in this process is lacking. This study addresses these gaps by employing scRNA-seq to elucidate the cellular heterogeneity and dynamic evolution of GC, providing novel insights into the molecular landscape of this malignancy.

Our analysis of the single cell dataset, which includes various stages of GC progression, revealed several key findings. As is known to all, the IM stage plays a critical role in the dynamic progression of GC. During this phase, gastric epithelial cells undergo transformation to resemble intestinal cells, marked by the expression of specific genes [43]. This process is often triggered by chronic inflammation, commonly due to *H.pylori* infection [44]. IM serves as a precancerous condition, setting the stage for further genetic and epigenetic alterations that lead to dysplasia and eventually invasive GC. Understanding the molecular and cellular changes during IM is crucial for early detection and prevention of GC [45, 46]. First, we identified distinct cell populations within gastric tissues, characterized by specific marker genes. *MUC6* and *MUC2* play critical roles in the progression from normal gastric mucosa to cancer. *MUC6* acts as a protective barrier and its downregulation is linked to increased cancer risk, while *MUC2* is a marker of IM and its upregulation indicates a higher risk of GC [47, 48]. Altered expressions of these mucins reflect epithelial changes and can serve as important biomarkers for early detection and potential therapeutic targets. In addition, *OLFM4*, a marker of intestinal stem cells, plays a significant role in tumorigenesis by promoting cell proliferation and inhibiting apoptosis. Its overexpression has been linked to various cancers, including gastric, colorectal, and pancreatic cancers, where it contributes to tumor growth, invasion, and poor prognosis [49–51]. *OLFM4*'s involvement in maintaining stem cell properties and its regulatory effects on key signaling pathways make it a potential biomarker and therapeutic target in cancer progression [52]. *OLFM4* is a novel marker of IM in gastric tissue has important guiding implications for the development of biomarkers for early cancer and prevention of IM progression. However, further validation in prospective, well-characterized human cohorts of GC is required to confirm its clinical applicability.

Investigating the process of gastric epithelial transformation to cancer is of critical importance as it provides insights into the molecular and cellular mechanisms driving gastric carcinogenesis. Understanding this progression helps identify key biomarkers and therapeutic targets, enabling early detection and more effective treatment strategies. Additionally, it elucidates the role of various genetic and environmental factors, such as *H.pylori* infection, in promoting tumor development, ultimately contributing to better prevention and management of GC. In this study, we combined with scRNA-seq and bulk data set identified is characterized by *UPP1* high expression of tumor cell subgroup. UPP1 is a key enzyme involved in pyrimidine metabolism, specifically in the reversible phosphorolysis of uridine and deoxyuridine to uracil and ribose-1-phosphate (R1P). *UPP1* plays a critical role in the salvage pathway of nucleotide synthesis, which is essential for maintaining the nucleotide pool balance in cells. This pathway is particularly important in rapidly proliferating cells, such as cancer cells, which have high demands for nucleotides to support DNA and RNA synthesis [25]. Our pseudotime analysis demonstrated that *UPP1* expression increases progressively from normal gastric epithelial cells to malignant GC cells. Functional assays confirmed that *UPP1* enhances GC cell proliferation and migration, suggesting its oncogenic role. In GC, *UPP1* has been implicated in promoting tumor growth and progression. The enzyme's ability to generate R1P through uridine catabolism provides an alternative energy source for cancer cells, supporting their survival and proliferation under metabolic stress conditions. This alternative pathway can complement glycolysis, allowing cancer cells to thrive even when glucose availability is limited. The upregulation of *UPP1* in GC is associated with poor prognosis, as it enhances the metabolic flexibility and adaptability of tumor cells. Understanding the regulatory mechanisms and functional roles of *UPP1* in cancer metabolism can offer new insights into potential therapeutic targets. Inhibiting *UPP1* activity may disrupt the metabolic balance of cancer cells, rendering them more vulnerable to existing treatments and improving clinical outcomes for patients with GC. The expression and prognosis of all genes above in GC were presented by GEPIA and Kaplan-Meier Plotter databases (Figure S2).

H.pylori infection is a critical factor in the progression from precancerous lesions to GC. It induces chronic inflammation, leading to atrophic gastritis, IM, and dysplasia. *H.pylori* promotes the production of reactive oxygen species (ROS) and pro-inflammatory cytokines, which cause DNA damage and genetic mutations. The

bacteria also activate oncogenic signaling pathways, such as NF-κB and Wnt/β-catenin, and inhibit tumor suppressor pathways like p53 [53]. Moreover, *H.pylori* 's virulence factors, such as CagA and VacA, contribute to cellular alterations and malignant transformation [54–56]. Thus, *H.pylori* infection significantly drives the multistep process of gastric carcinogenesis. The study by Yang et al. explores how *H.pylori* induces chronic atrophic gastritis through the IRF8-IFN-γ signaling axis. Incorporating the study by Yang, we emphasize that therapeutic targeting of inflammation-associated pathways can significantly reduce the progression of gastric diseases [57]. Further, we explored the impact of *H.pylori* infection on gene expression, revealing that *H.pylori* infection upregulates *UPP1* expression via the NF-κB pathway, specifically through P65 activation. This finding was corroborated by PCR experiments and bioinformatics predictions, which indicated that *P65* binds to the *UPP1* promoter, enhancing its expression in response to *H.pylori* -induced inflammation. Previous study highlights the significance of pyrimidine metabolism genes as prognostic biomarkers in gastric cancer. Integrating this knowledge with our analysis of *UPP1*'s role in glycolysis-related metabolic reprogramming emphasizes the clinical relevance of targeting pyrimidine metabolism pathways in gastric cancer treatment [58]. In addition, consistent with the findings of Skinner OS et al., *UPP1* can provide energy to cancer cells by breaking down uridine, under glucose-restricted conditions. Our findings align with and expand upon previous research that has identified *UPP1* as a key player in various cancers. The progressive increase in *UPP1* expression during GC development corroborates studies that have linked *UPP1* to tumor cell proliferation and migration. The role of *H.pylori* in gastric carcinogenesis has been well-documented, and our study provides a mechanistic link between *H.pylori* infection and *UPP1* upregulation via the NF-κB pathway, adding a new dimension to the understanding of *H.pylori* 's role in GC. Our study opens several avenues for future research. The role of *UPP1* in GC progression warrants further investigation, particularly in exploring its potential as a therapeutic target. Wang et al. provides evidence that *UPP1* is a novel immune-related target in brain glioma and that its high expression is associated with worse survival outcomes. While their study focuses on glioma, the implication that *UPP1* can modulate the immune microenvironment is particularly relevant to our findings. *UPP1*'s potential role in immune evasion mechanisms in gastric cancer, possibly through the modulation of pyrimidine metabolism and inflammatory pathways, warrants further exploration [59]. Future studies should focus on developing *UPP1* inhibitors and evaluating their efficacy in preclinical and clinical settings. Additionally, the interaction between *H.pylori* infection and *UPP1* expression via the NF-κB pathway suggests that targeting this inflammatory pathway could be a viable strategy for preventing or treating GC in *H.pylori* -infected individuals. Targeting *UPP1* could be an effective strategy for disrupting tumor metabolism, especially in tumors that are reliant on uridine salvage pathways. The development of *UPP1* inhibitors, or the combination of *UPP1* inhibition with current metabolic-targeted therapies, could offer new avenues for treatment. This is particularly relevant for cancers where *UPP1* is highly expressed and linked to poor prognosis, as evidenced by our analysis of gastric cancer datasets. Based on our findings, drugs that inhibit *UPP1* could be used in conjunction with other therapies. For instance, combining *UPP1* inhibition with drugs that target glycolysis or oxidative phosphorylation could block multiple metabolic pathways in tumors. While our study provides strong evidence for the role of *UPP1* in gastric cancer progression, we recognize that additional experimental validation, including in vivo models and clinical studies, is necessary to confirm the therapeutic potential of targeting *UPP1*. The identification of *UPP1* as a biomarker for gastric cancer and its relationship with metabolic and inflammatory pathways represents a novel area for future research, but its translation into clinical practice will require further investigation.

Additionally, our cell-cell communication analysis highlighted the significant role of the MIF pathway in modulating interactions, contributing to GC progression. Our study's detailed mapping of MIF interactions across different cell types provides a comprehensive view of its influence on GC dynamics, offering potential targets for therapeutic intervention. Our findings also highlight the importance of the MIF pathway in the inflammation-cancer transformation. Future research should aim to dissect the specific mechanisms by which MIF signaling influences inflammation-cancer transformation and explore the therapeutic potential of targeting MIF and its receptors. By mapping the interaction networks, we identified critical nodes and hubs where MIF signaling exerted significant influence, orchestrating a coordinated response that supports tumor progression. This was particularly evident in the interactions between cancer cells and gastric epithelial cells, where MIF signaling appeared to enhance inflammation-cancer transformation. Our study underscores the multifaceted role of the MIF pathway in the inflammation-cancer transformation. By delineating the specific contributions of MIF and its receptors in various cell populations, we provide a detailed understanding of how this signaling axis drives the dynamic evolution of GC. These insights offer valuable implications for therapeutic targeting, suggesting that disrupting MIF signaling could potentially impede tumor growth.

In summary, this study provides significant insights into the molecular and cellular mechanisms driving GC

progression. *MUC6*, *MUC2*, and *OLFM4* play crucial roles in the intestinalization process during gastric carcinogenesis. *MUC6*, a gastric mucin, is typically downregulated during IM, while *MUC2*, an intestinal mucin, is upregulated, indicating a shift towards an intestinal phenotype. *OLFM4*, a marker of intestinal stem cells, becomes increasingly expressed in metaplastic gastric cells, signifying a transformation towards an intestinal-like stem cell state. This transition is pivotal for the development of IM, a key precursor to GC, marking significant changes in cellular identity and function that drive tumorigenesis. The identification of *UPP1* as a key oncogenic factor, its regulation by *H.pylori* -induced NF-κB signaling, and the critical role of MIF-mediated cell-cell communication collectively enhance our understanding of gastric carcinogenesis. These findings have important implications for the development of novel therapeutic strategies targeting these pathways. However, further research is needed to validate these findings in larger cohorts and to explore their clinical applications. The integration of multi-omics approaches and advanced bioinformatics tools will be crucial in unraveling the complex molecular networks underlying GC, ultimately contributing to improved patient outcomes. While our study provides comprehensive insights into the dynamic evolution of GC, several limitations should be noted. The use of scRNA-seq data, while powerful, may not capture the full complexity of the dynamic process of tumorigenesis due to technical limitations and potential sampling biases. Additionally, our findings need to be validated in larger, independent cohorts to ensure their generalizability. Future studies should also incorporate longitudinal samples to better understand temporal changes in gene expression and cellular composition during GC progression. In conclusion, this study advances our understanding of the molecular mechanisms underlying GC and highlights potential targets for therapeutic intervention. By addressing the limitations and building on our findings, future research can pave the way for more effective strategies to combat this deadly disease.

Abbreviations

GC	Gastric cancer
scRNA-seq	Single-cell RNA sequencing
NAG	Non-atrophic gastritis
CAG	Chronic atrophic gastritis
IM	Intestinal metaplasia
EGC	Early gastric cancer
H.pylori	Helicobacter pylori
UPP1	Uridine phosphorylase 1
GEPIA	Gene Expression Profiling Interactive Analysis
GEO	Gene Expression Omnibus
PCs	Principal components
UMAP	Uniform manifold approximation and projection
DEGs	Differentially expressed genes
TCGA	The Cancer Genome Atlas
FBS	Fetal bovine serum
GMC	Gastric mucous cells
MIF	Macrophage migration inhibitory factor
ROS	Reactive oxygen species

Supplementary Information

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Supplementary Material 1

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Author contributions
All of the authors worked collaboratively on the work presented here. XC, BZ, SW, XJ and YD designed the experiments and supervised the study. SW, YP, JX, YL and MZ searched the articles and made figures; XC and FY wrote this manuscript. All authors read and approved the final manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval and consent to participate
This study was approved by the Ethics Committee of Affiliated Hospital of Yangzhou University (2022-YKL6-KE06). The study was performed in accordance with the Helsinki Declaration and Rules of Good Clinical Practice. All participants signed written informed consents after fully explained.

Consent for publication
We have obtained consent to publish this paper from all the participants of this study.

Competing interests
The authors declare no competing interests.

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