

SEC31A promotes gastric cancer progression by inducing the PI3K/AKT/mTOR pathway and enhancing the CCL5-mediated M2 polarization and recruitment of tumor-associated macrophages

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Abstract. SEC31 homolog A (SEC31A) is associated with a variety of cancers. This study elucidated the role of SEC31A in gastric cancer (GC). The expression of SEC31A in GC was analyzed using western blotting and immunohistochemistry. Functional experiments were performed to detect the impact of SEC31A on GC proliferation, migration and invasion. The PI3K inhibitor LY294002 was used to explore the regulation of the biological functions by SEC31A through the PI3K/AKT/mTOR pathway in GC cells. Co-culture of THP-1 (human leukemia cell line of monocytic origin) and GC cells was conducted to investigate the impact of SEC31A on the M2 macrophage polarization in tumor immune microenvironment. Our investigation observed elevated expression of SEC31A in both GC tissue and cell lines. Through functional studies, suppression of SEC31A was found to decrease epithelial-mesenchymal transition (EMT), proliferation, migration and invasion in GC cells. Overexpression of SEC31A led to activation of the PI3K/AKT/mTOR signaling cascade. The PI3K inhibitor LY294002 counteracted the increases in proliferation, migration, invasion and EMT triggered by SEC31A. Co-culture experiments involving THP-1 and GC cells indicated that SEC31A enhanced the recruitment and M2 polarization of macrophages via CCL5 within the GC tumor microenvironment (ME). To assess the influence of M2 macrophages on malignant behavior of GC cells, M2 macrophage-conditioned medium (M2-CM) was applied. M2-CM mitigated the inhibitory effects on malignant phenotypes in GC cells following SEC31A knockdown. Moreover, diminished SEC31A expression enhanced the efficacy of anti-PD-1

therapy in GC models. Collectively, SEC31A promoted GC progression via activating the PI3K/AKT/mTOR pathway and facilitating CCL5-mediated M2 polarization and recruitment of tumor-associated macrophages. SEC31A knockdown may augment the anti-tumor response to anti-PD-1 treatment in GC. These findings highlight SEC31A as a valuable prognostic indicator and potential target for immunotherapy in GC, offering novel strategies to enhance the effectiveness of anti-PD-1 therapy.

Introduction

Worldwide, gastric cancer (GC) is fifth in terms of occurrence among malignant tumors and is a principal factor in mortality associated with cancer, with an estimated global 968,000 new cases and 660,000 deaths in 2022 (1). The survival rate of advanced GC is low, but the survival rate of early treatment for gastric cancer is >90% (2,3). Therefore, it is key to identify early diagnostic markers and elucidate the potential biological mechanisms underlying GC progression.

SEC31 homolog A (SEC31A) serves as an external constituent of coat protein complex II, which is involved in coating vesicles that facilitate transport from the endoplasmic reticulum to the Golgi apparatus (4-6). Initial research has shown SEC31A is involved in endoplasmic reticulum membrane shaping for vesicle formation and in cargo molecule selection (7,8). The circular RNA of SEC31A promotes tumor progression in non-small cell lung cancer (9,10), whereas SEC31A is associated with a favorable prognosis in meningioma (11). It was hypothesized that SEC31A exhibits tumor type-dependent effects. However, the biological function and mechanism of action of SEC31A in GC remain unclear. PI3K/AKT/mTOR signaling participates in a variety of important processes of GC, such as apoptosis, autophagy and cell proliferation (12). To the best of our knowledge, there are no studies on the association between SEC31A and the PI3K/AKT/mTOR signaling pathway. Tumor-associated macrophages (TAMs) have served an important role in the progression of GC (13). To the best of our knowledge, however, the role of SEC31A in TAMs has not been reported.

The present study aimed to investigate the association between SEC31A expression and GC progression, its regulatory mechanisms in the development and progression of GC,

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the association between SEC31A and immune cell infiltration, as well as the impact of SEC31A on immunotherapy for GC, thereby providing new theoretical foundations for potential applications in the diagnosis and treatment of GC.

Materials and methods

Patients and samples. Specimens from GC patients were collected from Yantai Yuhuangding Hospital of Qingdao University, Yantai, China between April and December 2025, including 82 GC tissue samples and 50 matched (where possible) adjacent non-tumorous gastric tissue samples. A total of 82 patients were included. The cohort comprised 43 male and 39 female patients, with an age range of 30 to 85 years. The present study was approved by the Medical Ethics Committee of the Yantai Yuhuangding Hospital of Qingdao University (approval no. 2025-156). Inclusion criteria were as follows: i) Histopathologically confirmed diagnosis of gastric malignant tumor; ii) receipt of radical gastrectomy; iii) availability of comprehensive clinicopathological data; and iv) provision of written informed consent. The exclusion criteria were as follows: i) Prior history of other malignancies or previous gastric surgery; and ii) pre-specimen exposure to radiotherapy, chemotherapy or targeted therapy. Western blotting was performed on 30 pairs of GC and normal tissue. Immunohistochemistry (IHC) was performed on 20 paired tumor and adjacent non-tumorous tissue samples, along with 32 additional, unpaired tumor samples obtained from the hospital cohort, supplemented by commercially sourced tissue microarrays purchased from Shanghai Outdo Biotech Company (cat. nos. HStmA160CS01 and HStmAde060CS-01), which collectively comprised 52 GC specimens with complete clinical annotation. IHC assessment of SEC31A expression was conducted across the combined cohort of hospital-derived samples and tissue microarrays to examine the association between SEC31A expression and the clinicopathological characteristics of GC.

Bioinformatics analysis. Expression differences and survival analyses of SEC31A were obtained from The Cancer Genome Atlas (TCGA)-STAD dataset (14), GSE30727 dataset (15), GSE56807 dataset (16), GSE63089 dataset (17), TIMER2.0 database (cistrome.shinyapps.io/timer/) (18) and the Kaplan-Meier Plotter (kmplot.com/analysis/) for GC (19). The tumor mutational burden (TMB) analysis, microsatellite instability (MSI) analysis, gene set enrichment analysis (GSEA), Kyoto Encyclopedia of Genes and Genomes (KEGG, <https://www.kegg.jp/>) pathway enrichment analysis and immune characteristics analysis were performed using RStudio (version 2024.12.1, <https://posit.co/downloads>).

The TCGA-STAD dataset obtained 405 samples, of which 32 samples were normal gastric tissue samples and 373 samples were tumor samples. According to the median value of SEC31A expression, 373 patients in TCGA-STAD were divided into high expression group (187 cases) and low expression group (186 cases). The cutoff value was 66.8228. Univariate and multivariate Cox regression analysis were employed to identify the independent indicator of poor prognosis for those with GC.

KEGG enrichment analysis was used to examine the relationship between SEC31A expression level and related signaling pathways in GC. The correlation between SEC31A expression level and other genes was analyzed using TCGA-STAD dataset, and a gene set was obtained using $P < 1 \times 10^{-5}$ and Spearman correlation coefficient > 0.5 as screening conditions. Based on the aforementioned gene set, the clusterProfiler (version 4.16.0; <http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>) and KEGG.db (version 3.2.4; Bioconductor; <https://bioconductor.org/packages/3.11/data/annotation/html/KEGG.db.html>) packages and RStudio (version 2024.12.1; rstudio.com/products/rstudio/download/) were used for KEGG enrichment analysis.

To predict immunotherapy response, evaluations of Immunophenoscore (IPS) and Tumor Immune Dysfunction and Exclusion (TIDE) scores were performed between high and low expression group of patients with GC patients from TCGA-STAD cohort.

Literature review. To identify chemokines derived from GC cells that are known to recruit macrophages, a literature review was conducted from inception of the database to April 2026. The PubMed database (<https://pubmed.ncbi.nlm.nih.gov>) was searched using the following key term combinations: 'stomach neoplasms'[MeSH Terms] AND 'chemokines'[MeSH Terms] AND ['tumor associated macrophages'(MeSH Terms) OR 'macrophage activation'(MeSH Terms) OR 'macrophages'(MeSH Terms)]. The reference lists of identified key articles were examined to capture additional relevant studies. Inclusion criteria were: i) Studies demonstrating that the chemokine is expressed or secreted by GC cells; and ii) functional evidence showing that this chemokine contributes directly to macrophage recruitment or accumulation.

RNA isolation and reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from stably transfected GC and polarized THP-1 cells using TRIzol (Thermo Fisher Scientific, Inc.). RT was performed in compliance with the manufacturer's protocols using the SPARKscript II RT Plus kit (Shandong Sparkjade Scientific Instruments Co., Ltd.). RT-qPCR was performed with 2XSYBR Green qPCR Mix (Shandong Sparkjade Scientific Instruments Co., Ltd.) on a fluorescent quantitative PCR instrument (Shanghai Funglyn Biotechnology Co., Ltd.). Thermocycling conditions: Initial denaturation was carried out at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 15 sec, and extension at 72°C for 30 sec, concluding with a final extension step at 72°C for 5 min. Each experimental group consisted of three independent biological replicates, with each replicate assayed in triplicate. Relative expression of the target genes was calculated using the $2^{-\Delta\Delta C_q}$ method (20). The primers are detailed in Table S1.

Western blotting. Cell and tissue lysates were prepared on ice using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.) supplemented with 1X phosphatase inhibitors and 1x protease inhibitors and protein concentrations were quantified using the BCA Protein Assay Kit. A total of 20 μ g protein/lane underwent separation on SDS-PAGE

gels with 8-10% concentration and were transferred to polyvinylidene difluoride membranes. Membranes were blocked for 1 h at room temperature with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20, then incubated overnight at 4°C with primary antibodies (1:1,000). After that, membranes were incubated with secondary antibodies (1:5,000) for 1 h at room temperature. The primary and secondary antibodies are detailed in Table SII. Protein bands were visualized using a ChemiScope 6200 Touch Imaging System (Clinx Science Instruments Co., Ltd.) with ECL reagents (Shandong Sparkjade Scientific Instruments Co., Ltd.). Western blot band intensities were quantified using ImageJ software (version 1.53k; National Institutes of Health). Each experiment was independently repeated ≥ 3 times.

IHC and immunofluorescence. Tissues were fixed in 4% paraformaldehyde at room temperature for 24 h. Paraffin embedding was performed on the tissues, followed by sectioning to a thickness of 4 μ m. Paraffin-embedded sections were deparaffinized in xylene and rehydrated through a descending ethanol, after which antigen retrieval was performed by heating at 95-100°C for 30 min in citrate buffer (pH 6.0). Treatment with 3% H₂O₂ was then applied to inhibit endogenous peroxidase activity. Primary antibody incubation (1:200) overnight at 4°C was performed, followed by incubation with secondary HRP-labeled antibodies (1:500, Table SII) at room temperature for 1 h. Staining was performed using diaminobenzidine and nuclei were counterstained with hematoxylin at room temperature for 2 min. Each image was captured using a light microscope and analyzed via the ImageJ IHC Profiler (21), with scoring according to the H-score system (22).

Immunofluorescence was performed using the protocol for IHC. Tissues were incubated with anti-CD8a primary antibody (1:100) overnight at 4°C, followed by CoraLite488-conjugated secondary antibody (1:200; Table SII) for 1 h at room temperature. Nuclei were mounted using Antifade Mounting Medium containing DAPI (cat. no. P0131; Beyotime Biotechnology). Images were acquired using a fluorescence microscope. The fluorescence intensity was quantified using ImageJ software (version 1.53k; National Institutes of Health).

Cell culture and transfection. MKN74, AGS, MKN45, HGC27, GES-1, MFC and THP-1 cells (Table SIII) were subjected to short tandem repeat identification and mycoplasma detection. MKN74, MKN45, HGC27, MFC and GES-1 cells were maintained in RPMI-1640 (Shanghai VivaCell Biosciences, Ltd.), whereas AGS cells were cultured in F12K medium (Cellverse Co., Ltd.) at 37°C in a 5% CO₂ humidified incubator. All media were supplemented with 10% FBS (Shanghai VivaCell Biosciences, Ltd.), 100 U/ml penicillin and 100 μ g/ml streptomycin (Shandong Sparkjade Scientific Instruments Co., Ltd.). THP-1 cells were grown in THP-1-specific complete medium (Procell Biosciences).

For SEC31A knockdown, short hairpin (sh)RNA sequences targeting human SEC31A (Table SIV) were cloned into the GV493 vector (pFU-GW-016; Shanghai Genechem Co., Ltd.). A non-targeting shRNA (scramble) sequence was cloned into the vector to generate the knockdown control. For SEC31A overexpression, the full-length coding sequence of human SEC31A isoform 1 (RefSeq accession no. NM_001077207.4)

was cloned into the lentivirus vector plasmid GV492 (pGC-FU-GFP-Puro; Shanghai Genechem Co., Ltd.). The corresponding empty vector was used as the overexpression control.

Lentiviral particles for SEC31A gene knockdown and overexpression were produced using the second-generation lentiviral packaging system. 293T cells (Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd.) were co-transfected with 4 μ g transfer plasmid, 3 μ g packaging plasmid psPAX2 and 1 μ g envelope plasmid pMD2.G, using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.) at 37°C for 6 h, after which the medium was replaced with fresh complete medium. The viral supernatant was collected at 72 h post-transfection, pooled, filtered through a 0.45 μ m membrane, and stored at -80°C until use.

For lentiviral transduction, transduction with SEC31A gene knockdown and overexpression lentiviruses was performed on GC cells at ~50% confluency, applying a multiplicity of infection of 20. The transduction mixture was incubated with the cells at 37°C, 5% CO₂ for 12 h, after which the medium was replaced with fresh complete medium. At 48 h post-transduction, cells were subjected to selection with 1 μ g/ml puromycin for 72 h to eliminate non-transduced cells. The surviving cells were then maintained in standard culture medium with 100 U/ml penicillin and 100 μ g/ml streptomycin for an additional 72 h to recover before being used for subsequent functional assays. Transfection efficiency was confirmed by RT-qPCR and western blotting, as aforementioned.

Cell Counting Kit-8 (CCK-8) assay. Cells underwent stable transfection were plated into 96-well plates (1,000 cells/well for AGS and 800 cells/well for HGC27). Evaluation of cell proliferation was performed at 24, 48, 72, 96, 120 and 144 h) through CCK-8 assay (Dojindo Molecular Laboratories, Inc.). At each time point, 10 μ l of CCK-8 reagent was added per well, and after 1 h of incubation, absorbance was measured at 450 nm employing a microplate reader (Bio-Rad Laboratories, Inc.). Each experiment was independently repeated ≥ 3 times.

Colony formation assay. AGS and HGC27 cells were plated into 6-well plates at a density of 1,000 cells/well. The definition of colonies was a cluster of >50 cells. After culturing at 37°C for 14 days, the colonies were fixed at room temperature for 15 min in 4% paraformaldehyde solution, after which they were subjected to a 15 min staining with 1% crystal violet at room temperature. Images of the colonies were captured, and counting was performed utilizing ImageJ software (version 1.53k; National Institutes of Health). Each experiment was independently repeated ≥ 3 times.

Wound healing assay. Stably transfected AGS and HGC27 cells were seeded in a 6-well plate at a density of 8×10^5 cells/well. A 200 μ l pipette tip was used to generate a wound in the ~90% confluent cell monolayer. RPMI-1640 for HGC27 cells or F12K medium (Cellverse Co., Ltd.) for AGS cells containing 1% FBS was used. Microscopic images were obtained using a light microscope at both 0 and 36 h, with the wound gap quantified through measurements performed using ImageJ software (version 1.53k; National Institutes of Health). Each experiment was independently repeated ≥ 3 times.

Transwell invasion assay. Transwell invasion assays were performed using 24-well Transwell inserts with 8 μ m pore polycarbonate membranes. Upper chambers, pre-coated with Matrigel at 37°C for 1 h, were seeded with AGS cells at a concentration of 8×10^4 or HGC27 cells at 5×10^4 in 200 μ l serum-free F12K or RPMI-1640. The lower chamber was filled with medium enriched with 10% FBS. Following 48 h incubation at 37°C, the upper chamber was stained using 1% crystal violet at room temperature for 15 min. Images were captured using a light microscope and quantification of invading cells was performed through ImageJ software (version 1.53k; National Institutes of Health). Each experiment was independently repeated ≥ 3 times.

PI3K/AKT pathway inhibitor treatment. LY294002 (cat. no. HY-10108) was purchased from MedChemExpress. GC cells in vector and SEC31A overexpression group were treated at 37°C for 24 h with 20 μ M LY294002.

Macrophage differentiation. For M0 macrophage differentiation, THP-1 cells were stimulated with 100 ng/ml Phorbol 12-myristate 13-acetate (PMA, MedChemExpress) at 37°C for 24 h period. Induction of M1 polarization involved incubation of M0 macrophages with 20 ng/ml IFN- γ and 150 ng/ml lipopolysaccharide (LPS; MedChemExpress) at 37°C for 48 h. For M2 polarization, M0 macrophages were treated with 20 ng/ml IL-4 and 20 ng/ml IL-13 (MedChemExpress) at 37°C for 48 h. TAMs derived from AGS (TAM_{AGS}) and HGC27 (TAM_{HGC27}) cells were obtained by co-culturing M0 macrophages with AGS or HGC27 at 37°C for 48 h.

Macrophage migration assay. GC cells were incubated with RPMI-1640 medium without serum at 37°C for 48 h. The supernatant of incubated GC cells was collected into a 15 ml centrifuge tube. The supernatant was centrifuged at $1,500 \times g$ for 20 min at 4°C, and the supernatant was collected and filtered through a 40 μ m cell filter (Changde Beckman Biotechnology Co., Ltd.). Conditioned medium of GC cells was obtained by supernatant to 10% FBS. Macrophage migration assays were performed using 24-well Transwell inserts with 8 μ m pore polycarbonate membranes (cat. no. 3422; Corning, Costar). In a Transwell system, the lower chamber was filled with conditioned medium obtained from GC cells (10% FBS) with different levels of SEC31A expression, while 200 μ l serum-free RPMI-1640 medium was added to the upper chamber with 2×10^5 M0 macrophages. Following 24 h incubation at 37°C, macrophage migration was evaluated by staining the lower slide of the filters with 1% crystal violet at room temperature for 15 min. Images were captured using a light microscope and quantification of migration cells was performed through ImageJ software (version 1.53k; National Institutes of Health). Each experiment was independently repeated ≥ 3 times.

ELISA. The concentration of the secreted CCL5 protein in the culture medium supernatant was quantified using the CCL5 human ELISA kit (cat. no. JL11689, Jiangsu Jianglai Biotechnology Co., Ltd.) according to the manufacturer's instructions. Each experiment was independently repeated ≥ 3 times.

CCL5 treatment. CCL5 (cat. no. HY-P70450) was purchased from MedChemExpress. The GC cells in SEC31A-sh2 group were treated at 37°C for 24 h with 20 ng/ml CCL5.

In vivo tumorigenesis assay. The animal experiments were approved by the Medical Ethics Committee of the Yantai Yuhuangding Hospital of Qingdao University (approval no. K2026-498). In total, 12 male nude BALB/c mice (age, 5 weeks) were obtained from Shandong Pengyue Laboratory Animal Technology Co., Ltd. and randomly allocated into two experimental groups ($n=6$ /group) as follows: i) Scramble, injections of scramble control cells; and ii) SEC31A-sh2 group, injected with SEC31A-sh2-transfected cells. The mice were housed under specific-pathogen-free conditions at $22 \pm 2^\circ\text{C}$ and 50-60% relative humidity, with a 12-h light/dark cycle, and received a standard laboratory diet and water *ad libitum*. Subcutaneous administration of 5×10^6 HGC27 cells (150 μ l) was performed for each mouse weighing 20-22 g. Tumor volume was evaluated at 2-day intervals until a volume of $\sim 1,000 \text{ mm}^3$ was achieved, calculated as: Longest diameter $\times$ (shortest diameter) $^2 \times 0.5$. After a 21-day period, the mice were sacrificed. Humane endpoints were predefined as tumor volume $> 1,500 \text{ mm}^3$, tumor ulceration, necrosis or bleeding, impaired mobility, inability to access food or water, severe lethargy, persistent hunching, respiratory distress or body weight loss $> 20\%$ of baseline body weight. No mice required premature sacrifice according to our humane endpoints during this study. Subsequently, the xenograft tumors were photographed, measured, weighed and collected for additional analyses.

To investigate the impact of SEC31A on the efficacy of anti-PD-1 treatment in GC, C57BL/6 mice in each group ($n=5$ /group) that had been subcutaneously injected with MFC cells (5×10^6 cells, 150 μ l) were treated with anti-PD-1 antibody (clone no. RMP1-14, cat. no. #BP0146) or control IgG (both 10 mg/kg; both Bio X Cell; cat. no. #BP0089) twice/week from the 9th day after subcutaneous injection for 2 weeks. The four groups were as follows: Scramble + IgG group, SEC31A-sh3 + IgG group, Scramble + anti-PD-1 group, and SEC31A-sh3 + anti-PD-1 group. Stable transplantation of this tumor model has been established in previous studies (23-27). The method of administration was based on the previously described approach (28). After 23 days, the mice were sacrificed. Assessments of tumor dimensions and mass were conducted for each experimental group.

The sacrifice was conducted in accordance with established ethical and procedural guidelines (29,30). Mice received an intraperitoneal injection of sodium pentobarbital (150 mg/kg). Respiratory activity was monitored continuously following administration. Upon cessation of respiration, a 2 min observation period was implemented to ensure apnea. Absence of the corneal reflex was confirmed by tactile stimulation of the cornea. Cervical dislocation was performed to verify death.

Flow cytometry. Single-cell suspensions were prepared from tumor tissue, blocked with Purified Anti-Mouse CD16/32 (1:100; cat. no. E-AB-F0997A; Elabscience Biotechnology Co., Ltd.) at room temperature for 10 min, and stained with CD45-PE/Cyanine7 and CD8a-APC antibodies (Table SII). Detection was performed using a flow cytometer (MoFlo XDP; Beckman Coulter, Inc.). All raw data were analyzed

using FlowJo software (version 10.8.1; BD Biosciences). Each experiment was independently repeated ≥ 3 times.

Statistical analysis. Statistical analysis was performed using SPSS 29.0 (IBM Corp.) and GraphPad Prism 9.0 (Dotmatics) software. All data are expressed as the mean $\pm$ SD of ≥ 3 independent experiments. χ^2 tests compared categorical variable groups. In the case of continuous data, two-group differences were examined using paired or unpaired Student's t-test. Comparisons between multiple groups were analyzed by one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

SEC31A is upregulated in GC. To investigate SEC31A expression variations in GC, TIMER2.0, TCGA and GEO databases were used to compare its levels between GC and normal tissue. Results indicated significantly higher SEC31A mRNA expression in GC compared with normal samples (Fig. 1A-C). Western blot analysis of 30 paired GC and adjacent normal tissue samples showed significantly elevated SEC31A protein levels in GC samples (Fig. 1D and E). Consistent results were obtained through IHC analysis of 20 paired GC and adjacent tissue samples (Fig. 1F and G). As shown by RT-qPCR and western blotting, SEC31A expression was significantly upregulated in malignant cell lines (Fig. 1H-I).

Prognostic value of SEC31A in GC. Clinical characteristics of patients with GC were examined following stratification into high and low expression categories according to SEC31A levels. High SEC31A expression showed associations with advanced T, N and American Joint Committee on Cancer pathological stage (Table I). TCGA database survival data analysis revealed patients with higher levels of SEC31A expression showed significantly lower survival rates (Fig. S1A). This is consistent with the analysis based on the Kaplan-Meier Plotter database (Fig. S1B). Increased expression of SEC31A was an independent indicator of poor prognosis for those with GC (Fig. S1C and D). IHC was performed to gauge SEC31A expression in GC samples. Notably, the staining scores were significantly higher in samples from patients with GC with more advanced T and N stages, larger tumors and more advanced disease stages (Fig. S2A-D), while no significant associations were observed between H-score and age, sex or M stages (Fig. S2E-G).

Knockdown of SEC31A inhibits the proliferation, migration and invasion of GC cells in vitro. To assess SEC31A influence on GC cell biological functions, shRNAs were used to achieve SEC31A knockdown in AGS and HGC27 cells. The efficiency of SEC31A-sh1 and SEC31A-sh2 knockdown was verified by RT-qPCR and western blot analyses (Fig. 2A and B). SEC31A-sh1 and SEC31A-sh2 groups showed significantly decreased proliferation compared with the scramble control group (Fig. 2C and D). In wound healing assays, depletion of SEC31A markedly significantly the migratory capacity of HGC27 and AGS cells (Fig. 2E and F). Transwell invasion assays indicated that SEC31A knockdown suppressed the invasive capacity of these GC cell lines (Fig. 2G).

Knockdown of SEC31A inhibits the EMT and PI3K/AKT/mTOR signaling pathway. To explore the underlying mechanisms by which SEC31A modulates biological behaviors of GC cells, GSEA was performed using data obtained from TCGA database. The top five SEC31A-associated pathways are presented in Fig. S3A, among which 'epithelial mesenchymal transition' showed the strongest association (Fig. S3B). Cancer metastasis is influenced by EMT, which enhances migratory and invasive properties of tumor cells (31). Analysis of EMT-associated markers through western blotting demonstrated that SEC31A knockdown resulted in upregulation of E-cadherin while downregulating levels of N-cadherin and SNAI1 (Fig. 3A-C), providing evidence that SEC31A facilitated migration and invasion processes in GC cells. Correlation analysis using TCGA database identified a gene set significantly associated with SEC31A, which was filtered based on $P < 1 \times 10^{-5}$ and an absolute correlation coefficient > 0.5 . KEGG pathway analysis of this gene set revealed enrichment in 'PI3K-AKT signaling pathway' (Fig. S3C). SEC31A knockdown led to decreased phosphorylation of mTOR, AKT and PI3K, while the total levels of these proteins remained largely unchanged (Fig. 3D-F).

For verification of SEC31A function in GC progression under *in vivo* conditions, HGC27 cells were injected subcutaneously into nude mice. SEC31A knockdown HGC27 cells led to significantly reduced tumor development relative to the control (Fig. 3G-I). RT-qPCR demonstrated persistent SEC31A knockdown in the harvested tumors (Fig. 3J). Western blot analysis was performed on three randomly selected subcutaneous tumors from each group. Analysis of tumor specimens indicated significantly diminished phosphorylation levels of PI3K, AKT and mTOR in the SEC31A knockdown group, whereas expression of total proteins showed no significant alterations, aligning with *in vitro* observations (Fig. 3K and L). The remaining three tumors from each group were analyzed by IHC. The expression of Ki-67, a key marker associated with GC prognosis (32), was significantly decreased in tumors with SEC31A knockdown, as indicated by H-score (Fig. 3M and N). These findings indicate knockdown of SEC31A inhibited the PI3K/AKT/mTOR pathway in GC.

SEC31A regulates the proliferation, migration and invasion of GC cells through activation of the PI3K/AKT/mTOR pathway. To investigate whether SEC31A regulates the malignant phenotype of GC via the PI3K/AKT/mTOR pathway, SEC31A was overexpressed in GC cells and the efficiency of SEC31A overexpression was detected by RT-qPCR (Fig. S4A) and western blotting (Fig. S4B). SEC31A-overexpressing cells were treated with the PI3K/AKT inhibitor LY294002 to assess its effect on proliferation; decreased proliferation was observed in LY294002-treated SEC31A-overexpressing cells (Fig. 4A-D). Wound healing and Transwell invasion assays indicated that LY294002 counteracted the enhanced migratory and invasive capability resulting from SEC31A overexpression (Fig. 4E-H). LY294002 significantly reversed the SEC31A overexpression-mediated decrease in E-cadherin expression and elevation in phosphorylated (p-)AKT, p-mTOR and N-cadherin levels (Fig. 4I-K). In addition, the HGC27 cells of the vector and the SEC31A overexpression group were inoculated subcutaneously into nude mice and LY294002 was

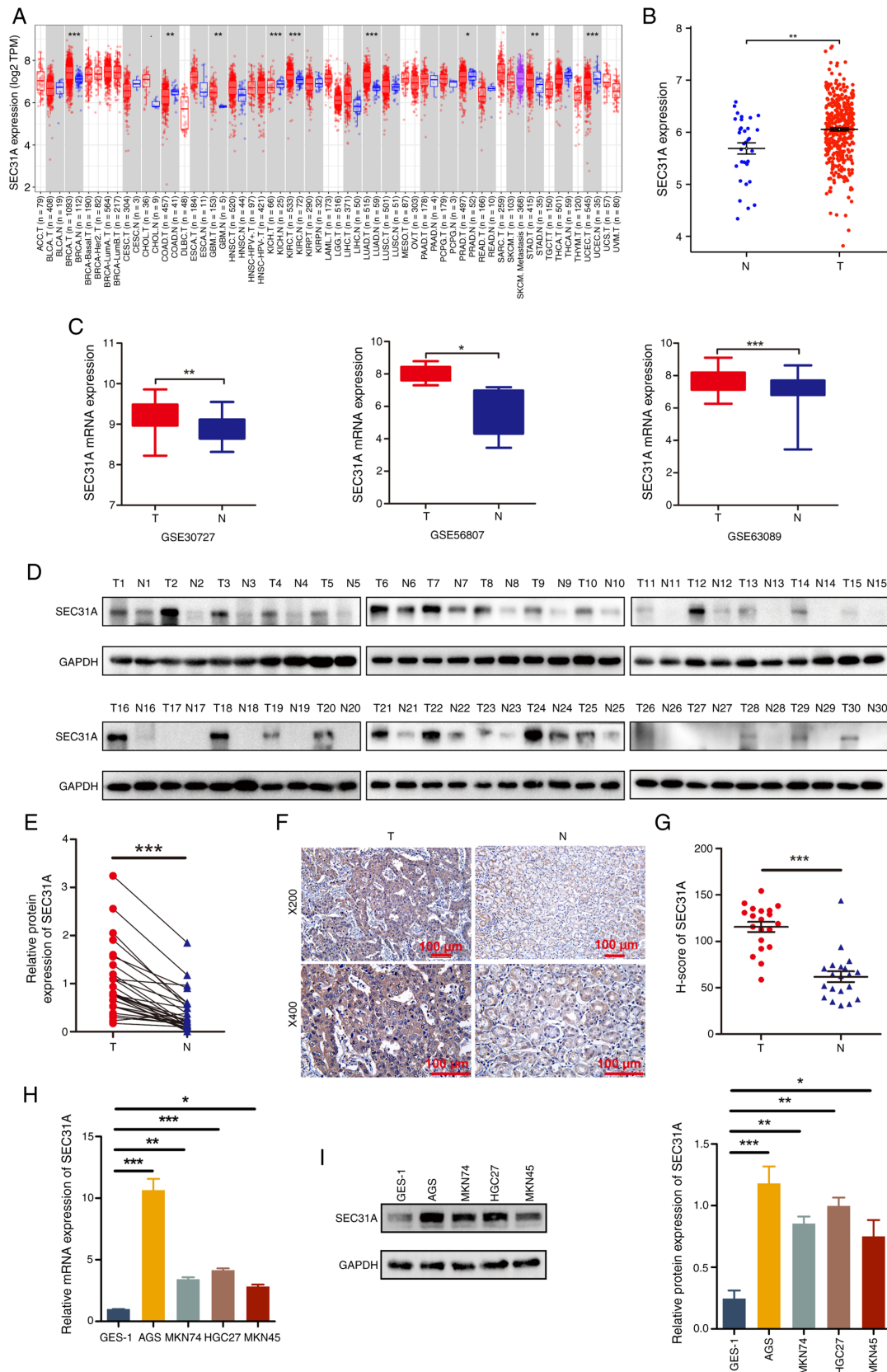


Figure 1. Elevated expression of SEC31A in GC specimens and cell models. (A) SEC31A transcript quantities in GC and adjacent non-malignant tissue samples. (B) Transcriptomic data from (B) TCGA database were used to compare SEC31A mRNA abundance between non-cancerous and GC specimens. (C) Transcriptomic data from GEO repositories were used to compare SEC31A mRNA abundance between non-cancerous and GC specimens. (D) Protein expression profiles of SEC31A in 30 matched pairs of GC and adjacent non-tumorous tissue were determined by western blot analysis. (E) Statistical analysis of western blot assay comparing SEC31A protein expression in GC tissues and adjacent non-tumorous tissues. (F) Immunohistochemical evaluation of SEC31A was performed on gastric T samples and N gastric mucosal tissues. (G) H-score measurements reflecting SEC31A immunoreactivity from malignant and matched N gastric tissue. (H) Using RT-qPCR, SEC31A mRNA expression was quantified across cell lines. (I) Via western blotting, SEC31A protein expression was quantified across cell lines. *P<0.05, **P<0.01 or ***P<0.001 vs. normal. GC, gastric cancer; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; RT-q, reverse transcription-quantitative; SEC31A, SEC31 homolog A; TPM, transcripts per million.

Table I. Association between SEC31A expression and clinical characteristics of patients with gastric cancer in The Cancer Genome Atlas.

Characteristic	Number of patients	Expression of SEC31A		P-value
		Low (n=186)	High (n=187)	
Sex (%)				0.490
Female	130 (34.8)	68 (18.2)	62 (16.6)	
Male	243 (65.1)	118 (31.6)	125 (33.5)	
Age, years (%)				0.068
≤65	172 (46.1)	77 (20.6)	95 (25.5)	
>65	201 (53.9)	109 (29.2)	92 (24.7)	
T stage (%)				0.042
T1/T2	97 (26.0)	57 (15.3)	40 (10.7)	
T3/T4	276 (74.0)	129 (34.6)	147 (39.4)	
N stage (%)				0.005
N0	119 (31.9)	72 (19.3)	47 (12.6)	
N1/N2/N3	254 (68.1)	114 (30.6)	140 (37.5)	
Distant metastasis (%)				>0.999
M0	350 (93.8)	175 (46.9)	175 (46.9)	
M1	23 (6.2)	11 (2.9)	12 (3.2)	
Pathological stage (%)				0.002
I/II	176 (47.2)	103 (27.6)	73 (19.6)	
III/IV	197 (52.8)	83 (22.3)	114 (30.6)	

SEC31A, SEC31 homolog A.

administered intraperitoneally. Overexpression of SEC31A promoted the growth of GC xenograft tumors, and LY294002 reversed the promoting effect of SEC31A on the growth of GC xenograft tumors (Fig. 4L-N). Collectively, these findings indicated that SEC31A facilitates proliferation, migration and invasion of GC cells by activating the PI3K/AKT/mTOR signaling cascade.

SEC31A promotes the M2 polarization of macrophages. SEC31A associations with the tumor immune ME (TIME) in GC were investigated using the TIMER2.0 database to assess correlations between SEC31A expression and immune cell infiltration levels. There was a positive association between SEC31A expression and infiltration of M2 macrophages (Fig. S5A). Tumors with elevated SEC31A levels showed increased M2 macrophages, inactive mast cells and dormant CD4⁺ memory T cells in the ME compared with low SEC31A expression tumors. Conversely, substantial decreases were observed in M0 macrophages, M1 macrophages, activated natural killer and activated mast cells (Fig. S5B). Additionally, examination of M2 biomarker expression within TCGA database demonstrated a marked positive correlation with SEC31A (Fig. S5C). These results indicated SEC31A may modulate the TIME by affecting macrophage polarization. THP-1 cells were subjected to PMA treatment to promote differentiation into M0 macrophages. These macrophages were co-cultured with AGS and HGC27 cells, resulting in the generation of TAM_{AGS} and TAM_{HGC27}, respectively. M1

polarization was induced by stimulating M0 macrophages with IFN- γ and LPS, while M2 macrophages were induced via IL-13 and IL-4. RT-qPCR was performed to assess the levels of CD80, IL-6, IL-10, CD163 and CD206 in M0, M1, M2, TAM_{AGS} and TAM_{HGC27} cells. M2-associated markers (IL-10, CD163 and CD206) were elevated in TAM_{AGS} and TAM_{HGC27}, whereas M1-associated markers (CD80 and IL-6) showed no significant changes compared with M0 macrophages (Fig. 5A). This suggested that TAM_{AGS} and TAM_{HGC27} exhibited characteristics more similar to those of M2 macrophages than M1 macrophages. To investigate the involvement of SEC31A in TAM polarization, GC cells with different SEC31A expression levels were co-cultured with M0 macrophages. RT-qPCR demonstrated that M2-associated markers were downregulated in TAMs co-cultured with SEC31A knockdown cells, while M1-associated markers remained largely unchanged (Fig. 5B and C). By contrast, RT-qPCR demonstrated that M2-associated markers were upregulated in TAMs co-cultured with SEC31A-overexpressing GC cells (Fig. 5D and E). Overall, these findings indicated that SEC31A promoted the M2 polarization of macrophages.

SEC31A promotes the M2 polarization and recruitment of macrophages via CCL5. To clarify whether SEC31A regulates the recruitment of macrophages, conditioned media from GC cells with SEC31A expression was used to assess the migratory capacity of macrophages. The findings demonstrated that the conditioned media of SEC31A-overexpressing

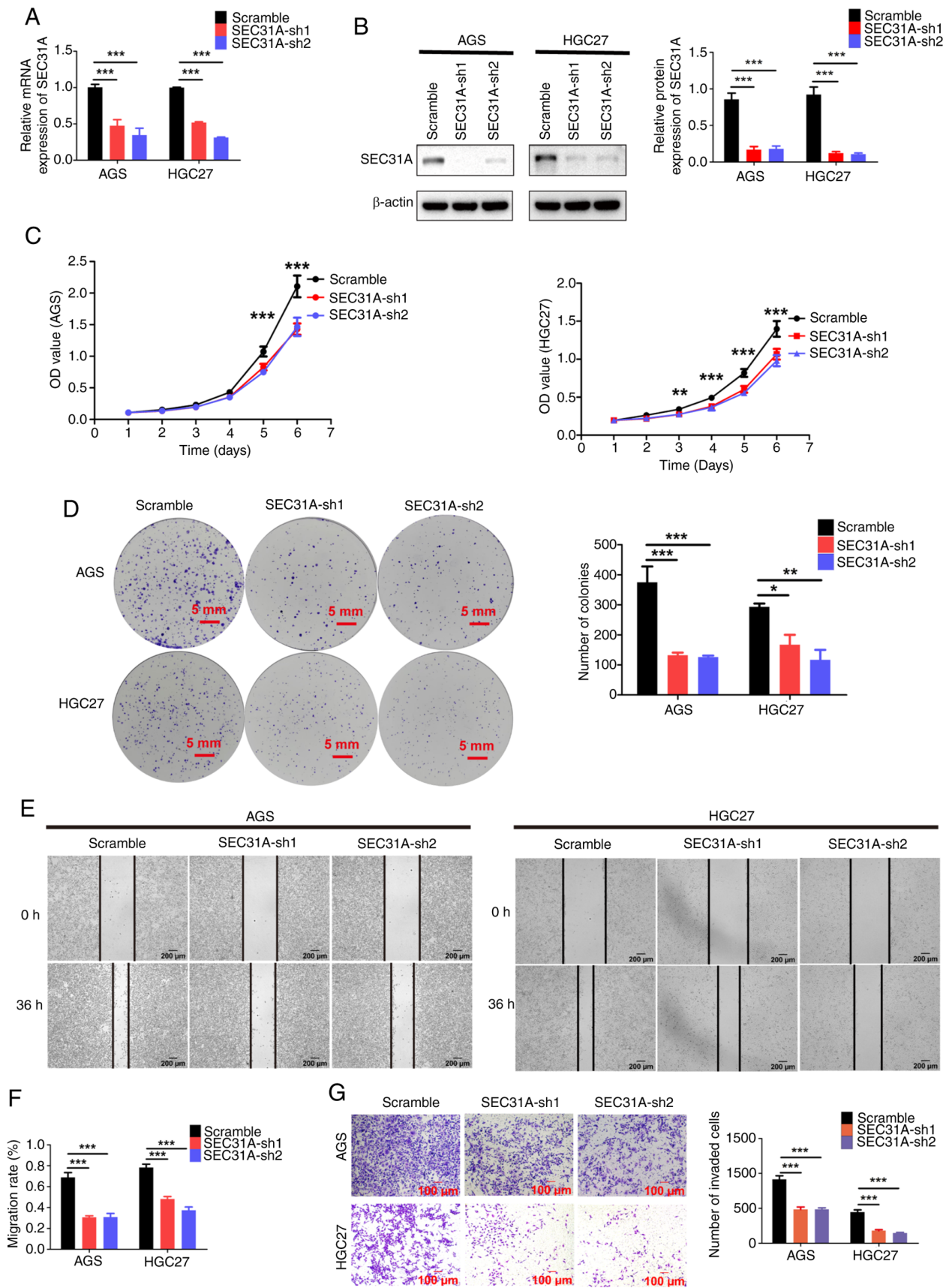


Figure 2. *In vitro* knockdown of SEC31A suppresses proliferation, migration and invasion in gastric cancer cells. (A) Efficiency of SEC31A knockdown was confirmed following transfection of AGS and HGC27 cells by reverse transcription-quantitative PCR. (B) Western blotting was employed to determine the knockdown efficiency of SEC31A in AGS and HGC27 cell lines. (C) Proliferative ability was assessed in GC cells following SEC31A knockdown. (D) Colony formation assays were performed to analyze the proliferative capacity of GC cells following SEC31A knockdown vs. scramble control. (E) Migration ability was evaluated in GC cells following SEC31A knockdown. (F) Statistical analysis of wound healing assays comparing migration rates of GC cells. (G) Invasion capacity was detected in GC cells following SEC31A knockdown, employing Transwell invasion assays. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$ vs. scramble. SEC31A, SEC31 homolog A; sh, short hairpin; OD, optical density.

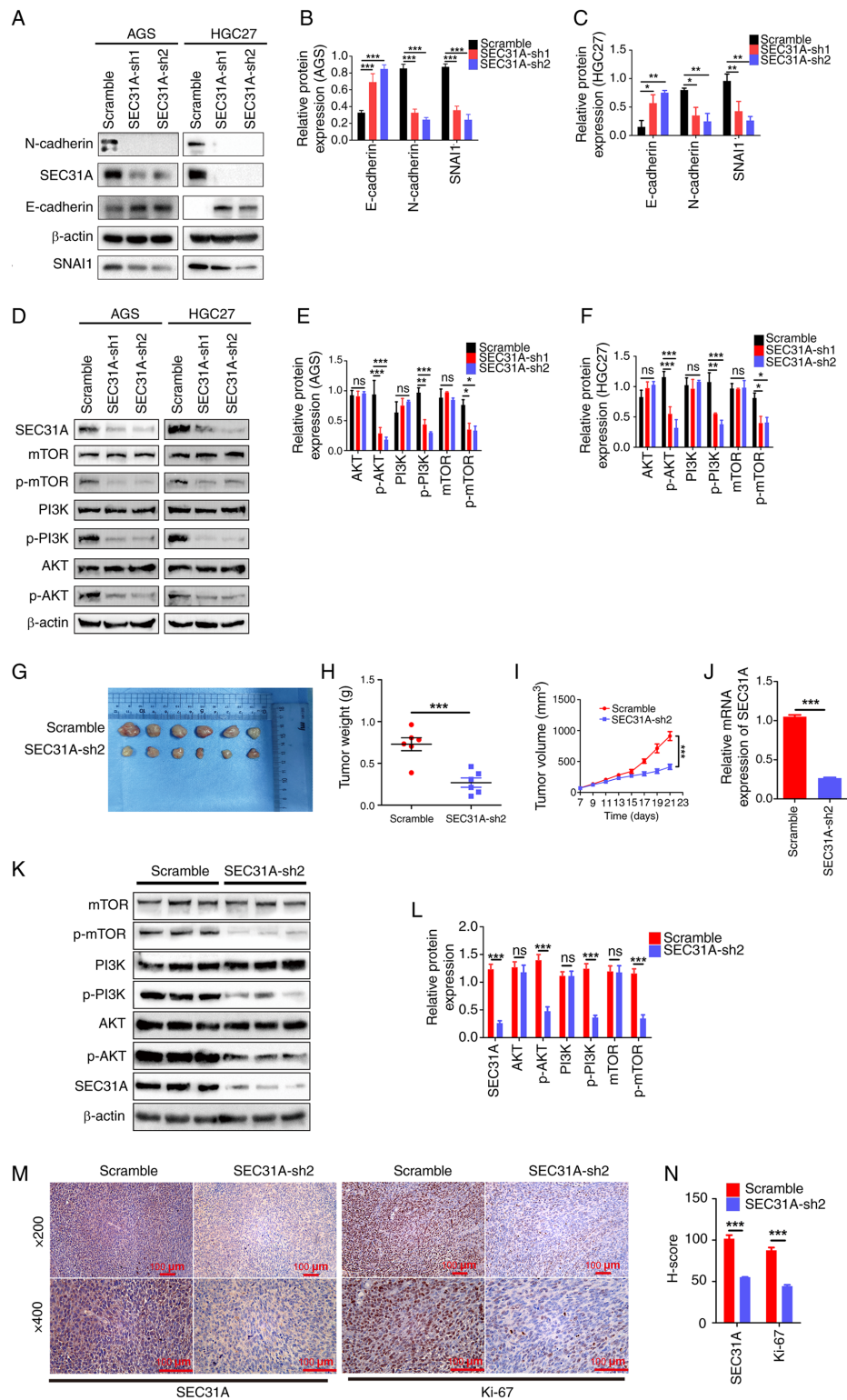


Figure 3. SEC31A knockdown leads to suppression of EMT and PI3K/AKT/mTOR signaling. (A) Western blot analysis was performed to assess how SEC31A knockdown affects EMT-associated markers. (B) Statistical analysis of EMT-related markers in AGS cells following SEC31A knockdown vs. scramble control, as assessed by western blotting. (C) Statistical analysis of EMT-related markers in HGC27 cells following SEC31A knockdown vs. scramble control, as assessed by western blotting. (D) Effect of SEC31A knockdown on the PI3K/AKT/mTOR signaling pathway in GC cells was examined through western blot assay. (E) Statistical analysis of PI3K/AKT pathway-associated markers in AGS cells after SEC31A knockdown. (F) Statistical analysis of PI3K/AKT pathway-associated markers in HGC27 cells after SEC31A knockdown relative to scramble control, based on Western blotting. (G) Representative tumor images from nude mice following injection of Scramble or SEC31A-sh2 HGC27 cells. (H) Measurements of subcutaneous tumor mass in nude mice implanted with Scramble or SEC31A knockdown HGC27 cells. (I) Measurement of subcutaneous tumor growth volume in nude mice bearing xenografts of Scramble or SEC31A knockdown HGC27 cells. (J) Reverse transcription-quantitative PCR was performed to detect the expression of SEC31A in xenograft tumors. (K) Western blot analysis was performed to quantify expression of SEC31A and components of the PI3K/AKT/mTOR signaling pathway in xenograft tumors. (L) PI3K/AKT pathway-associated protein expression in subcutaneous GC xenografts in nude mice. (M) Immunohistochemical evaluation of SEC31A and Ki-67 expression in xenograft tumor tissue. (N) H-score quantification of SEC31A and Ki-67 expression in subcutaneous tumor tissues via immunohistochemical detection. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$. EMT, epithelial-mesenchymal transition; ns, not significant; SEC31A, SEC31 homolog A; SNAI1, Snail Family Transcriptional Repressor 1; sh, short hairpin; p-, phosphorylated.

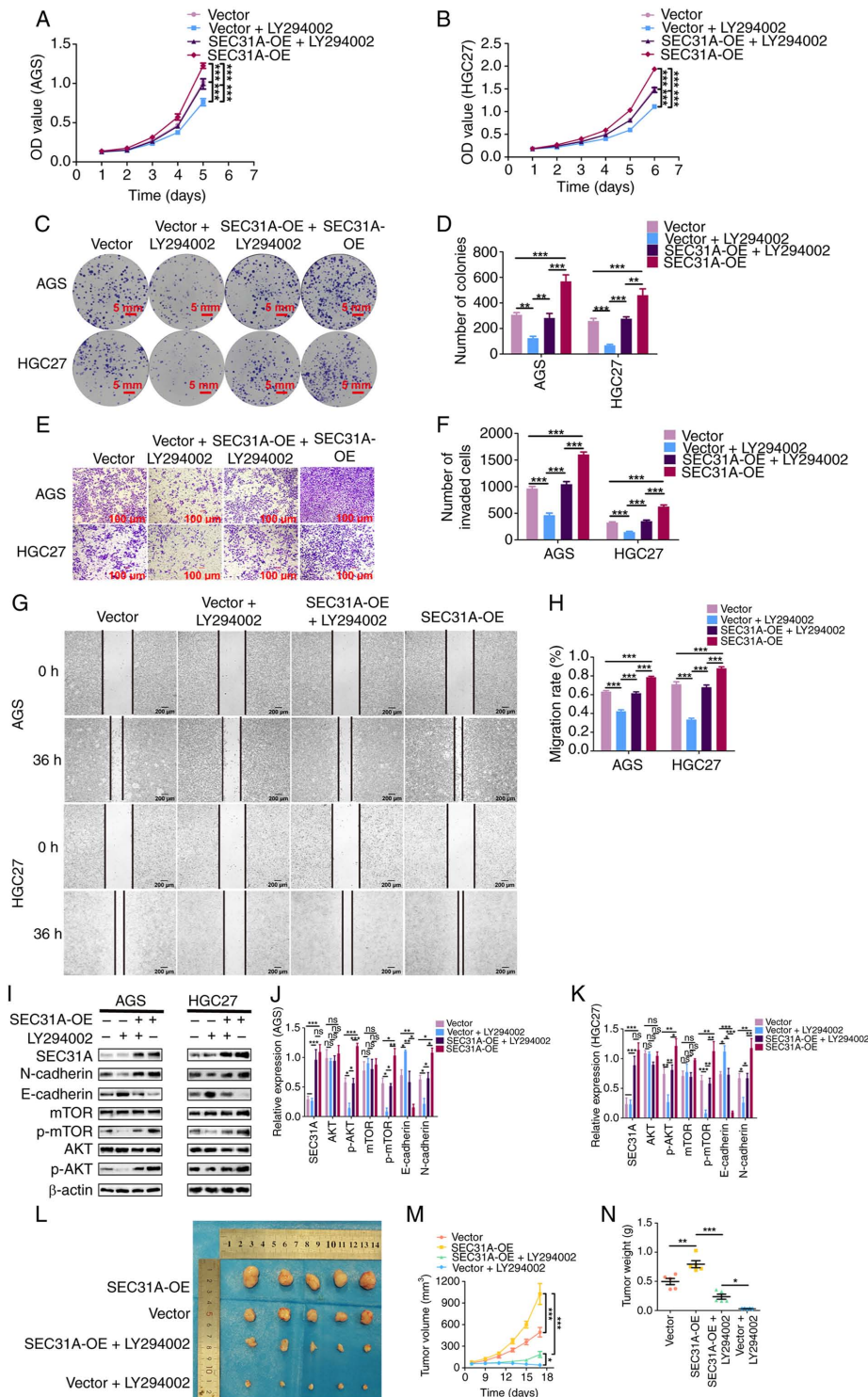


Figure 4. SEC31A influences proliferation, migration and invasion in GC cells via PI3K/AKT/mTOR pathway activation. (A) Proliferation was assessed using Cell Counting Kit-8 assay in AGS cells with SEC31A OE in the presence or absence of LY294002. (B) Cell proliferation was assessed in HGC27 cells following SEC31A OE, with or without LY294002 treatment, using the Cell Counting Kit-8 assay. (C) Cell proliferation was quantified in GC cells stably overexpressing SEC31A, both in the presence and absence of the LY294002, via the colony formation assays. (D) Statistical results of colony formation assays performed on GC cells stably overexpressing SEC31A, with or without LY294002-mediated PI3K inhibition. (E) Invasion ability of SEC31A-overexpressing GC cells in the presence or without LY294002 treatment is detected via Transwell invasion assays. (F) Statistical analysis of invasion capacity in GC cells overexpressing SEC31A, with or without LY294002 treatment. (G) Migration capacity was evaluated in GC cells with stable SEC31A OE in the presence or without LY294002 treatment, using wound healing assays. (H) Quantitative assessment of migration potential in GC cells with SEC31A OE, in the presence or absence of LY294002. (I) Effect of LY294002 on epithelial-mesenchymal transition-related markers was investigated via western blot analysis in cells overexpressing SEC31A. (J) Statistical analysis of the expression levels of EMT-related proteins in AGS cells with SEC31A OE in the presence or without LY294002 treatment. (K) Statistical analysis of EMT-related protein expression in HGC27 cells following stable SEC31A OE, with or without LY294002 administration. (L) Representative tumor images from nude mice following injection of vector or SEC31A OE HGC27 cells in the presence or without LY294002 treatment. (M) Measurements of subcutaneous tumor volume in nude mice implanted with vector or SEC31A OE HGC27 cells, under conditions with or without administration of LY294002. (N) Measurements of subcutaneous tumor mass in nude mice implanted with vector or SEC31A OE HGC27 cells, with or without LY294002 treatment. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$. GC, gastric cancer; ns, not significant; SEC31A, SEC31 homolog A; OD, optical density; OE, overexpression; p-, phosphorylated.

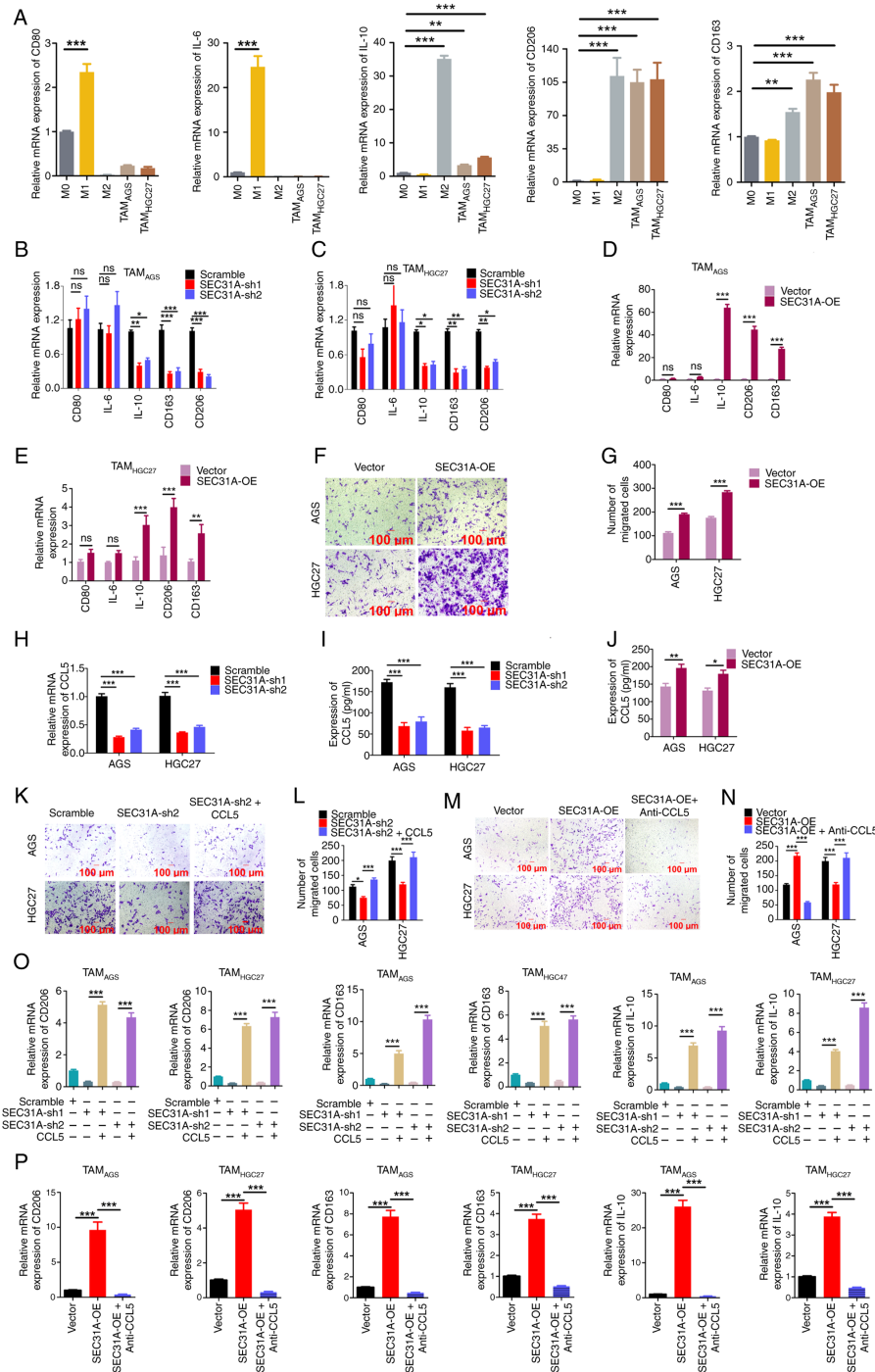


Figure 5. SEC31A promotes M2 polarization and recruitment of macrophages via CCL5. (A) Verification of M0, M1, M2 macrophages and TAMs was performed by RT-qPCR. (B) RT-qPCR was performed to detect the mRNA expression levels of CD80, IL-6, IL-10, CD163, and CD206 in TAMs of AGS cells in the Scramble group and the SEC31A knockdown group. (C) RT-qPCR analysis was conducted to quantify the mRNA expression levels of CD80, IL-6, IL-10, CD163, and CD206 in TAMs co-cultured with HGC27 cells transfected with either Scramble or SEC31A shRNA. (D) RT-qPCR was performed to detect the mRNA expression levels of CD80, IL-6, IL-10, CD163, and CD206 in TAMs of AGS cells in the vector group and the SEC31A OE group. (E) RT-qPCR was performed to detect the mRNA expression levels of CD80, IL-6, IL-10, CD163, and CD206 in TAMs of HGC27 cells in the vector group and the SEC31A OE group. (F) Transwell migration assays were performed to examine how SEC31A affects recruitment of macrophages. (G) Comparison of the effects of conditioned media from GC cells in the vector group and the SEC31A OE group on the migration capacity of TAMs towards the lower chamber of Transwell. (H) RT-qPCR was performed to analyze the CCL5 expression in GC cells with SEC31A knockdown. (I) ELISA was performed to detect the CCL5 levels in the supernatant of GC cells following SEC31A knockdown. (J) CCL5 was quantified in the culture supernatant of GC cells overexpressing SEC31A using ELISA. (K) Transwell migration assay was performed to evaluate the impact of CCL5 on the migratory capacity of macrophages treated with conditioned medium from GC cells with SEC31A knockdown. (L) Effects of conditioned medium from GC cells in the Scramble group, conditioned medium from GC cells in the SEC31A-sh2 group, and conditioned medium from GC cells in the SEC31A-sh2 group supplemented with CCL5 on the migration capacity of TAMs towards the lower chamber of the Transwell system. (M) Transwell migration assay was used to evaluate the impact of CCL5-neutralizing antibody on the migratory capacity of macrophages treated with conditioned medium from GC cells with SEC31A OE. (N) Effects of conditioned medium from GC cells on the migration capacity of TAMs towards the lower chamber of the Transwell system. (O) RT-qPCR was performed to assess the impact of CCL5 on the M2 polarization of TAMs in GC cells following SEC31A knockdown. (P) RT-qPCR was performed to assess the impact of CCL5-neutralizing antibody on the M2 polarization of TAMs in GC cells following SEC31A OE. *P<0.05, **P<0.01 or ***P<0.001. TAM, tumor-associated macrophage; RT-q, reverse transcription-quantitative; GC, gastric cancer; ns, not significant; SEC31A, SEC31 homolog A; sh, short hairpin; OE, overexpression.

GC cells enhanced the migration ability of macrophages (Fig. 5F and G). These results indicate that SEC31A could promote the accumulation of macrophages. To investigate the mechanism by which SEC31A facilitates the recruitment of macrophages, the present study performed a review of the literature regarding chemokines promoted by GC cells for the recruitment of macrophages. Chemokines CCL2 (33,34), CCL4 (35), CCL5 (36), CCL15 (37), CCL26 (38), CXCL1 (39), CXCL2 (40), CXCL8 (41), CXCL12 (42) and CX3CL1 (42) derived from GC cells recruit macrophages. RT-qPCR detected the expression of the aforementioned chemokines in GC cells of Scramble and SEC31A knockdown group. Knockdown of SEC31A decreased the expression of CCL5 (Fig. 5H), and the results of ELISA were also consistent with this (Fig. 5I). Conversely, ELISA demonstrated that the expression of CCL5 was upregulated in the conditioned medium of GC cells with SEC31A overexpression (Fig. 5J).

To explore whether SEC31A promotes the recruitment of macrophages by regulating CCL5 expression, GC cell conditioned medium (Scramble, SEC31A-sh2 group, SEC31A-sh2 + CCL5 group) was used. CCL5 reversed the reduced migration of macrophages caused by SEC31A knockdown (Fig. 5K and L). By contrast, CCL5 knockdown abolished the augmented macrophage migration induced by conditioned medium from SEC31A-overexpressing GC cells (Fig. 5M and N). As CCL5 serves a role in promoting the M2 polarization of macrophages (43), it was hypothesized SEC31A promotes the M2 polarization of macrophages via CCL5. RT-qPCR showed CCL5 reversed the decreased expression of IL-10, CD163 and CD206 in TAMs caused by SEC31A knockdown in GC cells (Fig. 5O). Neutralization of CCL5 reversed the M2 polarization of TAMs induced by SEC31A overexpression in GC cells (Fig. 5P).

SEC31A promotes GC malignancy by facilitating the recruitment of M2 macrophages. To investigate the role of M2 macrophage in the malignant properties of GC cells, M2 macrophage-conditioned medium (M2-CM) was used to culture SEC31A-knockdown GC cells. CCK-8 experiments revealed that M2-CM enhanced the proliferation of SEC31A-knockdown GC cells (Fig. 6A and B). According to wound healing and Transwell invasion assays, the diminished migratory and invasive potential of GC cells induced by SEC31A knockdown was effectively rescued by treatment with M2-CM (Fig. 6C-G). These findings suggested that M2 macrophages enhance the decreased malignant phenotypes induced by SEC31A knockdown. In summary, SEC31A promoted GC malignancy by facilitating the recruitment of M2 macrophages.

Knockdown of SEC31A enhances the efficacy of anti-PD-1 in the treatment of GC models in vivo. The association between SEC31A expression and TMB, MSI and TIDE score was examined. SEC31A expression was negatively associated with both TMB and MSI in GC (Fig. S6A and B). The TIDE score of patients with GC in the high SEC31A group was higher than that in the low expression group (Fig. S6C), while the IPS was lower (Fig. S6D). These findings suggested that patients with elevated SEC31A levels may have a poor response to treatment with immune checkpoint inhibitors. Anti-PD-1 therapy is a notable component of immune checkpoint inhibitor therapy.

As M2-type TAMs largely limit the efficacy of PD-1/PD-L1 blockade therapy in patients with GC (44), the present study investigated whether reprogramming TAMs by knocking down SEC31A could enhance the therapeutic effect of anti-PD-1 in GC models. To investigate the impact of SEC31A on anti-PD-1 therapy for GC, the present study intraperitoneally administered anti-PD-1 antibodies or IgG to C57BL/6 mice that had been subcutaneously inoculated with MFC cells from the Scramble or the SEC31A knockdown group. The efficiency of SEC31A knockdown in MFC cells was examined by RT-qPCR and western blotting (Fig. 7A-C). Tumor growth in the SEC31A-sh3 + anti-PD-1 group was markedly diminished in comparison with the SEC31A-sh3 + IgG and Scramble + anti-PD-1 groups (Fig. 7D-F). This suggested that SEC31A knockdown increases the responsiveness of GC models to anti-PD-1 treatment.

To investigate the effect of SEC31A on the infiltration of TAMs in GC models, IHC was performed to detect the expression of CD206 in subcutaneous tumors. Compared with the Scramble and Scramble + anti-PD-1 group, the expression of SEC31A and CD206 in subcutaneous tumors of the SEC31A-sh3 + IgG and SEC31A-sh3 + anti-PD-1 groups was significantly downregulated (Fig. 7G-I), indicating knockdown of SEC31A in GC models could significantly inhibit the infiltration of M2-type TAMs and the sole use of anti-PD-1 treatment did not affect the infiltration of M2-type TAMs. IF showed knockdown of SEC31A and anti-PD-1 treatment could increase the proportion of CD8-positive cells in tumor tissue and the knockdown of SEC31A combined with anti-PD-1 treatment increased the proportion of CD8-positive cells (Fig. 7J and K). Flow cytometry showed knockdown of SEC31A and anti-PD-1 treatment promoted the infiltration of CD8⁺ T cells in subcutaneous tumors. In addition, compared with knockdown of SEC31A and anti-PD-1 treatment, the knockdown of SEC31A + anti-PD-1 treatment could further increase the infiltration of CD8⁺ T cells in tumors (Fig. 7L and M). This indicated that knocking down SEC31A can activate CD8⁺ T cells by inhibiting the infiltration of M2-type macrophages, thereby enhancing the efficacy of PD-1 blockade therapy in GC models.

Discussion

To date, several studies have explored the involvement of SEC31A in different types of tumors (9-11). SEC31A promotes proliferation, migration and invasion in non-small cell lung cancer cells (9,10). Conversely, elevated SEC31A expression is associated with improved prognostic outcomes in meningioma (11), suggesting tumor type-dependent functional diversity of SEC31A. SEC31A overexpression, confirmed via database analysis and experimental results, was associated with poor clinical outcomes in GC. Distinct from the oncogenic role of SEC31A in lung cancer, the present study demonstrated its function in promoting immune escape via remodeling the immunosuppressive tumor ME (specifically M2 polarization) in GC, providing a rationale for combinatorial targeting strategies.

EMT is characterized by a decrease in E-cadherin and increase in N-cadherin levels (45,46). SNAIL is a key inducer of EMT (47,48). EMT enhances tumor cell migration and

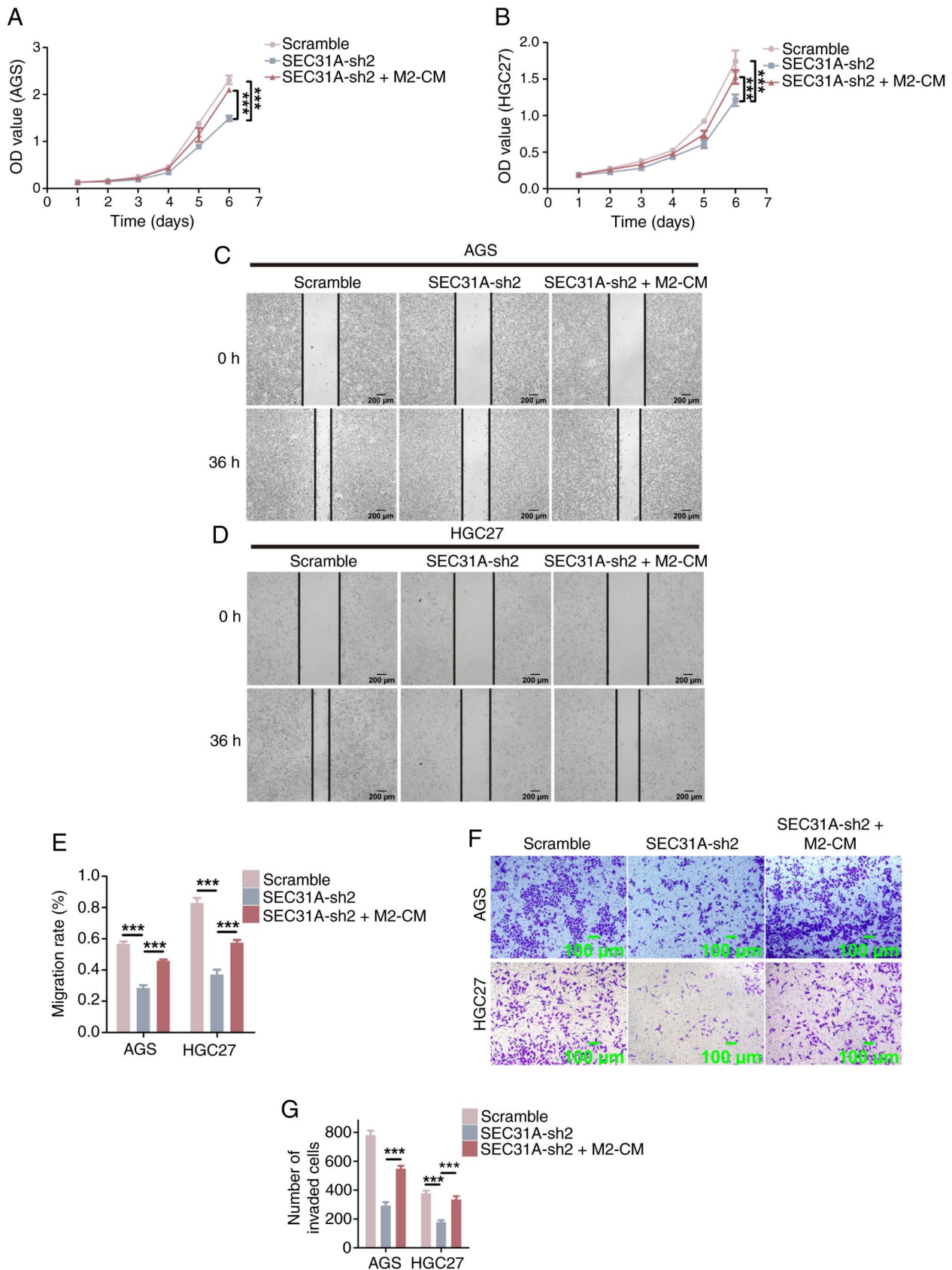


Figure 6. SEC31A enhances malignancy in GC through facilitation of M2 macrophage recruitment. (A) Proliferation capacity of SEC31A knockdown AGS cells was assessed via Cell Counting Kit-8 assay following co-culture with M2-CM. (B) Proliferative activity of HGC27 cells with stable SEC31A knockdown was evaluated using the Cell Counting Kit-8 assay after co-culturing with M2-CM. (C) Migration capability was evaluated using wound healing assays in SEC31A knockdown AGS cells cultured with M2-CM. (D) Migration was assessed by wound healing assays in HGC27 cells with stable SEC31A knockdown following exposure to M2-CM. (E) Migration rates of GC cells. (F) Invasion capability was evaluated using Transwell invasion assays in SEC31A knockdown GC cells cultured with M2-CM. (G) Number of invaded GC cells in the Scramble group, SEC31A knockdown group, and SEC31A knockdown + M2-CM treatment group compared via Transwell invasion assays. ***P<0.001. GC, gastric cancer; M2-CM, M2 macrophage-conditioned medium; SEC31A, SEC31 homolog A; OD, optical density; sh, short hairpin.

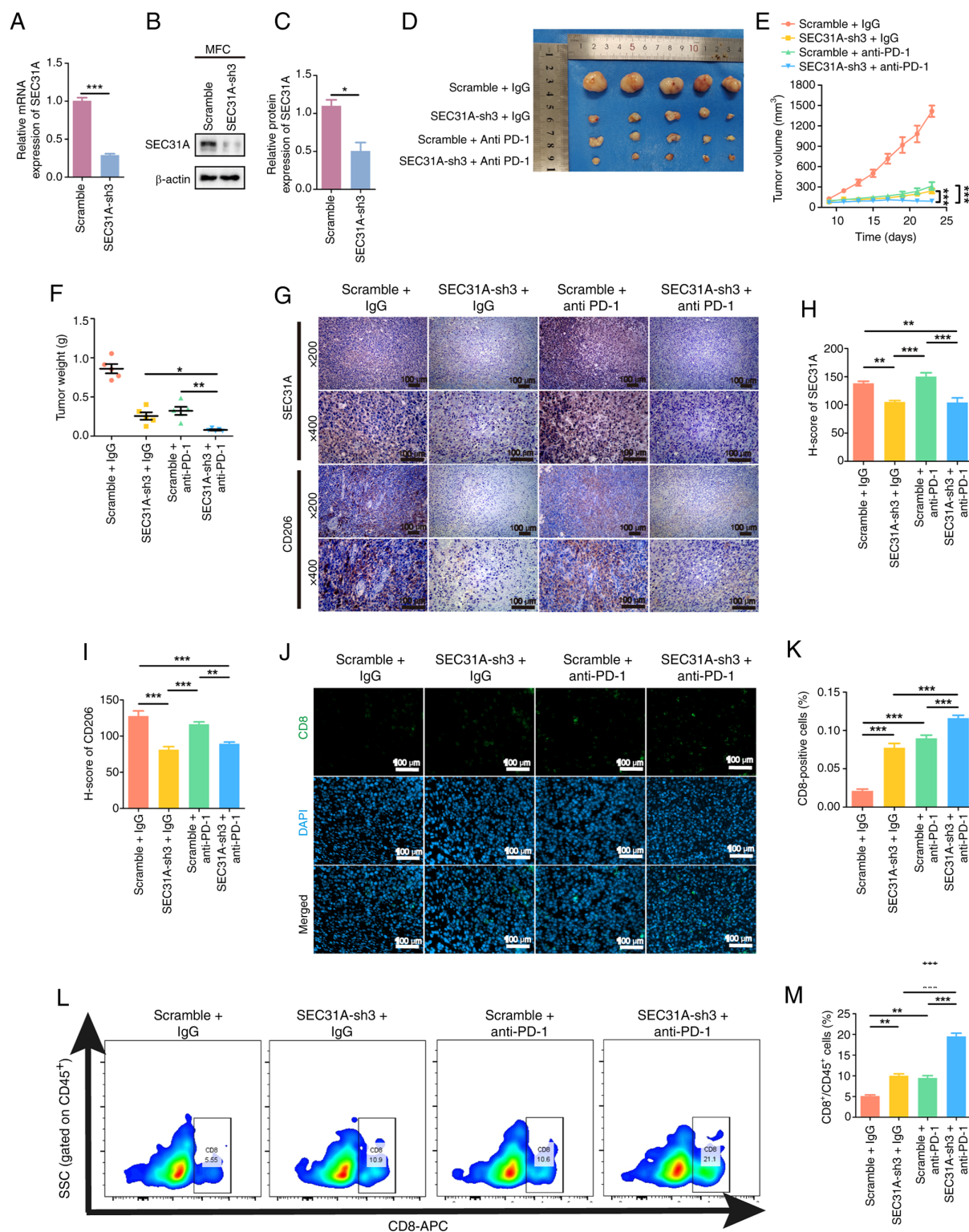


Figure 7. Knocking down SEC31A improves the efficacy of anti-PD-1 therapy in syngeneic gastric cancer models. (A) Efficiency of SEC31A knockdown was confirmed following transfection of MFC cells by reverse transcription-quantitative PCR. (B) Western blotting was performed to determine the knockdown efficiency of SEC31A in MFC cells (C) SEC31A expression in MFC cells following SEC31A knockdown vs. scramble control, as assessed by western blotting. (D) Representative tumor images from mice following subcutaneous injection of Scramble or SEC31A knockdown MFC cells, with IgG or anti-PD-1 treatment. (E) Subcutaneous tumor volume in mice implanted with Scramble or SEC31A knockdown MFC cells, under conditions with IgG or anti-PD-1 administration. (F) Measurements of subcutaneous tumor mass in mice implanted with Scramble or SEC31A knockdown MFC cells, with IgG or anti-PD-1 treatment. (G) Effect of SEC31A on the expression of CD206 in subcutaneous transplanted tumors of mice was detected by immunohistochemistry. (H) H-score quantification of SEC31A expression in subcutaneous tumor tissues in Scramble + IgG group, SEC31A-sh3 + IgG group, Scramble + anti-PD-1 group, and SEC31A-sh3 + anti-PD-1 group, via immunohistochemical detection. (I) H-score quantification of CD206 expression in subcutaneous tumor tissue. (J) Effect of SEC31A on CD8 expression in subcutaneous transplanted tumors of mice was detected by immunofluorescence. (K) CD8-positive cells percentages in subcutaneous tumor tissues in Scramble + IgG group, SEC31A-sh3 + IgG group, Scramble + anti-PD-1 group, and SEC31A-sh3 + anti-PD-1 group, via immunofluorescence. (L) Effect of SEC31A on the proportion of CD8⁺/CD45⁺ cells in subcutaneous transplanted tumors of mice was detected by flow cytometry. (M) Statistical results of the proportion of CD8⁺/CD45⁺ cells in subcutaneous tumor tissues in Scramble + IgG group, SEC31A-sh3 + IgG group, Scramble + anti-PD-1 group, and SEC31A-sh3 + anti-PD-1 group, via flow cytometry. * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$. SEC31A, SEC31 homolog A; sh, short hairpin.

invasion, thereby contributing to tumor metastasis (49-51). While previous studies have shown that SEC31A facilitates lung cancer metastasis (9,10), to the best of our knowledge, its role in EMT regulation had not been previously demonstrated. The present study showed that SEC31A promotes EMT in GC cells, suggesting that SEC31A may contribute to GC metastasis by driving EMT progression.

The PI3K/AKT/mTOR signaling cascade is a key regulatory mechanism governing tumor proliferation and metastatic processes (12,52), with its activation facilitating EMT across multiple types of cancer (53-55). Experimental analyses verified that the PI3K/AKT/mTOR pathway was inhibited in AGS and HGC27 cells following SEC31A knockdown. These results suggest that SEC31A enhances proliferative, migratory, invasive and EMT capabilities in GC cells, partially through PI3K/AKT/mTOR pathway activation. This was validated by experiments using the PI3K inhibitor LY294002 in SEC31A-overexpressing GC cells.

Other coat protein complex II vesicle proteins, such as SEC23, SEC24 and SEC13, are primarily involved in the physical deformation of the endoplasmic reticulum membrane into vesicles and selection of cargo molecules for their transport to the Golgi complex, regulating protein transport from the endoplasmic reticulum to the Golgi apparatus (56). SEC23 is associated with cell cycle, EMT and MAPK signaling (57-59). SEC24 regulates ferroptosis and TGF- β signaling (60,61). SEC13 is implicated in the regulation of the EMT and NF- κ B signaling (62,63). SEC31A has classical secretory and non-classical signal regulation functions (6,9). The present study showed that SEC31A participates in the regulation of PI3K/AKT/mTOR signaling. SEC31A targets the PI3K/AKT/mTOR pathway, forming a regulatory axis. To the best of our knowledge, whether SEC31A differs from other family members and specifically acts on the PI3K/AKT/mTOR pathway has not been confirmed and requires further validation.

TAMs, which are macrophages infiltrating neoplastic tissue, serve key roles in modulating tumor proliferation and metastatic processes. TAM polarization refers to the differentiation of TAMs into M1 or M2 phenotypes in response to signals from the surrounding ME (64,65). M1 macrophages secrete pro-inflammatory cytokines including IL-6, IL-12, IL-23 and tumor necrosis factor, which mediate pro-inflammatory, cytotoxic and anti-tumor effects. By contrast, M2 macrophages predominantly secrete cytokines such as IL-10 and TGF- β , which exert anti-inflammatory and tumor-promoting effects (66). The majority of TAMs display the M2 phenotype in GC (67). This phenotype facilitates processes such as angiogenesis, lymphangiogenesis, immunosuppression and hypoxia, along with tumor proliferation and metastasis, thereby demonstrating an association with unfavorable outcomes in multiple types of cancer (68-70), including GC (71), colorectal cancer (72) and hepatocellular carcinoma (73). Consistently, the present study showed that TAM_{AGS} and TAM_{HGC27} cells were more inclined towards the M2 phenotype.

Chemokines exert diverse effects on TAMs within tumors, such as facilitating the recruitment of TAMs to tumor immune cells, inducing the generation and infiltration of TAMs in tumors, promoting the proliferation, enhancing the tumor-promoting function, sustaining the inhibitory function

on antitumor immunity and regulating the polarization of TAMs (74). In the present study, SEC31A in GC cells was demonstrated to facilitate the recruitment and M2 polarization of macrophages by regulating the expression of CCL5 within the TIME of GC. CCL5 regulates the recruitment of TAMs and M2 polarization in a variety of tumor types (43,75,76), such as breast cancer (43), chordoma (75) and lung adenocarcinoma (77). SEC31A promotes the malignant progression of pancreatic cancer cells by modulating the secretion of C-X-C motif chemokine ligand 12 (CXCL12) in tumor-associated fibroblasts (78). To the best of our knowledge, the present study is the first report on the regulatory effect of SEC31A on CCL5.

M2 macrophages reversed the decreased malignant phenotypes of GC cells induced by SEC31A knockdown. M2 macrophages promote the progression of GC through CXCL8 activation of C-X-C motif chemokine receptor 1/2 on the membrane of GC cells (41), which is consistent with the present findings. The aforementioned results suggest that SEC31A promotes GC progression by enhancing M2 macrophage polarization and recruitment.

PD-1 and CTLA4 inhibitors are key therapeutic options for advanced GC (79-81). MSI and TMB status are potential biomarkers for predicting responses to immune-checkpoint inhibitors across multiple cancer types (82,83), such as colorectal cancer (82,83), GC (82,83) and melanoma (82,83). Previous studies have reported that patients with GC exhibiting elevated TMB and MSI scores tend to derive greater therapeutic benefit from immune-checkpoint inhibitors (84-86). The TIDE scoring model outperforms PD-L1 expression and TMB in predicting immunotherapy response to CTLA4 and PD-1 inhibitors (87). Elevated TIDE score is associated with enhanced resistance to therapy using immune checkpoint inhibitors (88). The present study demonstrated that SEC31A expression was inversely correlated with TMB and MSI score in GC, but was positively associated with TIDE scores. These findings suggested patients exhibiting elevated SEC31A levels may respond less favorably to immunotherapy involving immune checkpoint inhibitors.

Recent studies have identified TAMs as promising therapeutic targets for cancer (89-91). Corresponding therapeutic strategies primarily include eliminating M2 subsets, reprogramming TAMs to the anti-tumor M1 phenotype and blocking oncogenic signaling driven by TAMs (92). M2 polarization of TAMs in the TIME suppresses the responsiveness of tumors to anti-PD-1 treatment (93,94). Serine protease inhibitor clone E member 1 promotes the immune evasion of GC by increasing the expression of PD-L1⁺ M2 macrophages and enhancing the sensitivity of GC xenografts to anti-PD-1 treatment. The triad motif-containing protein 32/phosphodiesterase 9A pathway promotes immune escape in GC by inducing TAM recruitment and M2 polarization and impedes the effectiveness of anti-PD-1 therapy (28). *In vivo* experiments, knockdown of SEC31A can enhance the sensitivity of GC models to anti-PD-1 therapy. This suggests expression of SEC31A in GC influences the response to anti-PD-1 treatment. M2 macrophages exert an inhibitory effect on CD8⁺ T cells (95-98). The physical barrier formed by TAMs restricts the infiltration of CD8⁺ T cells into the TIME (95). TAMs promote the exhaustion of CD8⁺ T cells via upregulating PD-L1 expression (96),

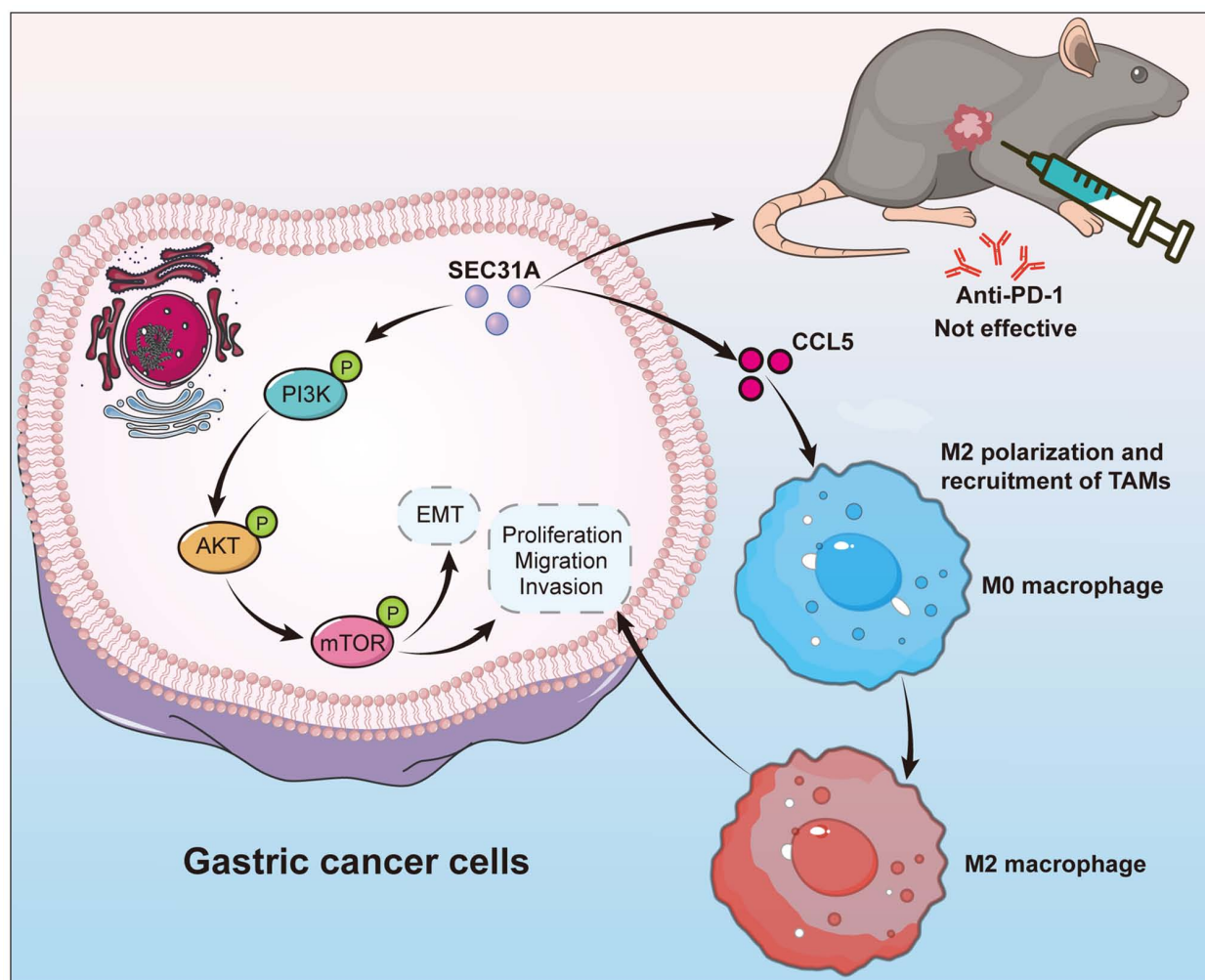


Figure 8. SEC31A promotes gastric cancer progression by inducing the PI3K/AKT/mTOR pathway and enhancing the CCL5-mediated M2 polarization and recruitment of TAMs. SEC31A enhances the resistance effect of PD-1 blockade in GC models. SEC31A, SEC31 homolog A; EMT, epithelial-mesenchymal transition; TAM, tumor-associated macrophage.

secreting immunosuppressive cytokines (97) and depriving T cells of the metabolites required for proliferation (98). As the present study confirmed that the expression of SEC31A in GC cells can regulate the M2 polarization of TAMs and SEC31A knockdown inhibited the infiltration of M2-type TAMs and promoted the infiltration of CD8⁺ T cells, it was hypothesized that SEC31A in GC inhibits the infiltration of CD8⁺ T cells in the TIME by inducing the M2 polarization of TAMs, resulting in resistance to anti-PD-1 therapy. The specific regulatory mechanism requires further verification.

Although the present study identified the role of SEC31A in the malignant progression of GC and modulating the TIME, as well as regulatory mechanisms, several limitations remain. The present study did not fully elucidate the specific regulatory mechanisms by which SEC31A modulates the M2 polarization of TAMs and influences the sensitivity to anti-PD-1 therapy. Furthermore, the present study relies on animal models and the development of SEC31A-targeting agents is warranted for clinical trial validation.

The present study demonstrated that SEC31A contributes to GC progression and TIME modulation (Fig. 8). Increased SEC31A levels in GC samples and cells were associated with poor clinical outcomes. Functionally, SEC31A enhanced

proliferative, migratory, invasive and EMT capacity in GC cells through PI3K/AKT/mTOR pathway activation. Additionally, SEC31A modulated immune infiltration profiles in GC tumors by promoting CCL5-mediated M2 macrophage polarization and recruitment, thereby promoting GC progression. SEC31A knockdown potentiated the efficacy of anti-PD-1 therapy in GC models. These results clarify SEC31A oncogenic functions in GC, and position it as a potential target for precision immunotherapeutic intervention.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XZ performed experiments, conceived the study, analyzed data, constructed figures and wrote and edited the manuscript. LH performed experiments, conceived the study, analyzed data, constructed figures and wrote and edited the manuscript. XZ and LH confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Medical Ethics Committee of the Yantai Yuhuangding Hospital of Qingdao University (Yantai, China; approval no. 2025-156). All patients provided written informed consent for participation. The animal experiments were approved by the Ethics Committee of Yantai Yuhuangding Hospital of Qingdao University, Yantai, China (approval no. K2026-498).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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