

Association of the CXCL–ACKR1 Signaling Axis with the Angiogenic Microenvironment in Endometrial Cancer: A Single-Cell Transcriptomic Analysis

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Purpose: To investigate the role of the CXCL signaling pathway in angiogenesis of endometrial cancer and evaluate its potential as a therapeutic target.

Patients and Methods: Single-cell RNA sequencing (scRNA-seq) data from 18 endometrial cancer and normal control tissues were integrated with The Cancer Genome Atlas (TCGA) database. Data were analyzed through clustering and cell type annotation to explore the tumor microenvironment, followed by cell–cell interaction analysis to assess the activity of the CXCL signaling pathway. We then quantified CXCL pathway activity using CellChat-derived ligand–receptor communication metrics (communication probability/interaction strength) and evaluated supporting gene expression differences for key ligands and receptor across tumor versus control samples.

Results: ScRNA-seq revealed a complex tumor microenvironment composed of epithelial, stromal, immune, and endothelial cells. The CXCL signaling pathway was significantly activated in endometrial cancer. Dominant signaling axes such as CXCL8–ACKR1, CXCL2–ACKR1, and CXCL3–ACKR1 were identified. CXCL8, CXCL2, and CXCL3 were highly expressed in tumor tissues and were predicted to interact with ACKR1 expressed on endothelial cells, suggesting a potential role of the CXCL–ACKR1 axis in regulating tumor-associated angiogenic signaling.

Conclusion: This study support an association between the CXCL–ACKR1 axis and endothelial-targeted signaling patterns consistent with angiogenesis in endometrial cancer. ACKR1 may serve as a promising anti-angiogenic therapeutic target, offering new insights into precision treatment strategies for endometrial cancer.

Keywords: endometrial cancer, ACKR1, CXCL signaling pathway, angiogenesis, single-cell RNA sequencing, tumor microenvironment

Introduction

Endometrial cancer is the most common gynecologic malignancy in developed countries, with incidence steadily increasing worldwide due to aging populations and rising obesity rates. It primarily affects postmenopausal women, with the average age at diagnosis around 60 years. Risk factors include obesity, diabetes, hypertension, nulliparity, and unopposed estrogen exposure. Incidence is higher in high-income regions such as North America and Europe compared to developing countries, though rates are also rising in Asia.¹ Early-stage diagnosis is common due to symptomatic abnormal uterine bleeding, contributing to generally favorable survival outcomes compared with other gynecologic cancers. However, late-stage patients have a lower five-year survival rate, with stage III ranging from 47% to 58% and stage IV from 15% to 17%.² Therefore, research on endometrial cancer still needs to be strengthened.

Angiogenesis refers to the process of new blood vessel formation from pre-existing vessels under the stimulation of angiogenic factors. Under normal physiological conditions, it plays a role in embryonic development, wound healing, and the menstrual cycle. However, in endometrial carcinoma, it provides significant support for tumor growth and metastasis.³ Endometrial carcinoma cells secrete various cytokines that promote angiogenesis, inducing endothelial cell proliferation, migration, and the formation of new blood vessels, thereby supplying nutrients and oxygen to the tumor while inhibiting cancer cell apoptosis. Angiogenesis not only promotes tumor growth but also suppresses apoptosis of cancer cells, leading to tumor recurrence and metastasis. Stromal cells and immune cells within the tumor microenvironment also play crucial roles in the angiogenic process.⁴ Although angiogenesis has been widely recognized as a critical process in tumor progression, the molecular mechanisms regulating angiogenic signaling in endometrial cancer remain incompletely understood. Most previous studies have primarily focused on classical angiogenic factors such as vascular endothelial growth factor (VEGF), while the broader network of chemokine-mediated signaling within the tumor microenvironment has received comparatively less attention. In particular, the cell-type-specific interactions between tumor cells, stromal cells, and endothelial cells that drive angiogenesis in endometrial cancer remain poorly characterized. Future research into the molecular mechanisms of angiogenesis and the identification of new therapeutic targets is expected to provide more effective strategies for the treatment of endometrial carcinoma.⁵ While classical pro-angiogenic mediators such as VEGF have been extensively studied in endometrial cancer, angiogenesis in this disease occurs within a tumor microenvironment that is also strongly shaped by inflammatory signaling. Chemokines of the CXC family are key coordinators of inflammation and vascular responses, as they can be produced by tumor and stromal compartments and influence endothelial behavior directly or indirectly through recruitment of myeloid cells.

Chemokines of the CXC ligand (CXCL) family have been increasingly recognized as important regulators of tumor angiogenesis and immune cell recruitment within the tumor microenvironment. Several CXCL chemokines, including CXCL8, CXCL2, and CXCL3, have been reported to promote endothelial cell proliferation, migration, and vascular remodeling in multiple malignancies. ACKR1 (Atypical Chemokine Receptor 1), also known as the Duffy antigen receptor for chemokines, is predominantly expressed on endothelial cells and has been shown to modulate chemokine gradients and vascular signaling. Emerging evidence suggests that interactions between CXCL chemokines and ACKR1 may influence tumor-associated angiogenesis, although their potential role in endometrial cancer remains largely unexplored.

Single-cell RNA sequencing (scRNA-seq), as an emerging sequencing technology, is a high-throughput technique that captures and analyzes the whole transcriptome information at the single-cell level. It can reveal the true differences and heterogeneity between cells, making it of significant value in cancer research.⁶ This method not only allows for the identification of different subpopulations of tumor cells, such as invasive cells, drug-resistant cells, and dormant cells, but also provides in-depth insights into the mechanisms of non-tumor components in the tumor microenvironment, such as immune cells, fibroblasts, and endothelial cells. This helps to uncover the interactions between tumors and the surrounding environment, as well as the pathogenesis, providing a powerful tool for understanding cancer development and optimizing precision treatment strategies.^{7–9} Currently, in studies on scRNA-seq in endometrial cancer, the sample sizes are limited in individual studies, which may introduce certain biases in the research findings. Furthermore, few studies have explored the mechanisms of angiogenesis. Therefore, this article intends to integrate scRNA-seq^{10–12} from multiple endometrial cancer samples and normal control samples to investigate the role of angiogenesis in endometrial cancer. We hypothesized that chemokine-mediated intercellular communication plays an important role in regulating tumor-associated angiogenesis in endometrial cancer. Through cell–cell interaction analysis, we aimed to identify key ligand–receptor signaling axes involved in angiogenic regulation, with particular attention to the CXCL–ACKR1 pathway. Understanding these interactions may provide new insights into the mechanisms of tumor angiogenesis and reveal potential therapeutic targets for endometrial cancer.

Materials and Methods

Data Collection

The scRNA-seq data of 10 endometrial adenocarcinoma tissues (including SRR17165223, SRR17165224, SRR17165229, SRR19842866, SRR19842869, GSM5276933, GSM5276934, GSM5276935, GSM5276936, GSM5276937) and 8 normal control endometrial tissues (including SRR19842871, SRR19842870, SRR17165231, SRR17165230, SRR17165227, GSM5572238, GSM5572239, GSM5572240) were downloaded from the Gene Expression Omnibus (GEO) database. The bulk gene expressions were downloaded from The Cancer Genome Atlas (TCGA) databases. All the datasets examined in this study are available through prior publications or can be accessed in the public domain, and thereby no relevant ethical approval is required. Due to the use of publicly available datasets, detailed clinical and pathological information (eg., tumor grade, stage, BMI, and hormonal status) was not consistently available across all samples. Therefore, downstream analyses were conducted based on the available transcriptomic data without stratification by these clinical variables.

Data Integration and Batch Effect Correction

To integrate scRNA-seq data from multiple public GEO datasets and minimize batch effects, we applied the standard Seurat integration workflow (Seurat v4.4.0). After quality control and normalization of individual datasets, highly variable genes were identified, and integration anchors were determined using the FindIntegrationAnchors function. The datasets were then integrated using the IntegrateData function, generating a batch-corrected expression matrix for downstream analyses.

Normalization of ScRNA-Seq Data

The Seurat R package was utilized for quality control and subsequent bioinformatics analysis. Cells were filtered based on the following criteria: nFeature_RNA between 200 and 3000, and mitochondrial gene percentages below 10%. After quality control, gene expression values were normalized using the LogNormalize method implemented in Seurat. This approach normalizes the gene expression counts for each cell by the total expression, multiplies the result by a scaling factor of 10,000, and performs logarithmic transformation to stabilize variance across cells. After quality control filtering based on gene number (200–3000 features) and mitochondrial gene content (<10%), a total of 65362 cells (1837 in Control 1, 1586 in Control 2, 3941 in Control 3, 3415 in Control 4, 2793 in Control 5, 679 in Control 6, 605 in Control 7, 638 in Control 8; 2558 in EEC 1, 8500 in EEC 2, 4360 in EEC 3, 1879 in EEC 4, 5796 in EEC 5, 5694 in EEC 6, 6384 in EEC 7, 1473 in EEC 8, 4285 in EEC 9, 8939 in EEC 10) were retained for downstream analysis.

Data Scaling and Dimensionality Reduction

The data was scaled to ensure genes with highly expressed genes do not dominate downstream analyses using the ScaleData function. We applied linear dimensionality reduction using principal component analysis (PCA) to capture major sources of variation in the dataset. Specifically, PCA was conducted on the top 2,000 highly variable genes, which were selected to represent the most informative features while minimizing noise from lowly expressed or non-informative genes.

Cell Clustering

After dimensionality reduction, cells were clustered using the Louvain algorithm implemented in the Seurat FindClusters function with a resolution parameter of 1.0, which resulted in the identification of 30 distinct cell subclusters. The FindClusters function of Seurat was used to process the normalized data.¹³ t-SNE was applied for nonlinear dimensional reduction to visualize the scRNA-seq data.

Identification of Marker Genes and Cell Annotation

Cell type annotation was performed using a sequential approach. Machine-assisted annotation was first applied to obtain preliminary cell type classifications, which were then validated and refined based on canonical marker genes curated in

the CellMarker 2.0 database. This combined, sequential approach improved the accuracy and reliability of cell type identification. EPCAM and KRT8 are marked for epithelial cells; DCN and COL1A1 are for stromal cells; VWF and PECAM1 are for endothelial cells; CD3D, CD3E and CD3G are for T cells; CD68 and CD163 for macrophages; NKG7, GNLY and KLRF1 are for NK cells; ARHGAP15, SKAP1 and ITK are for NKT cells; PDGFRB and NOTCH3 for pericytes; CD79A and MZB1 for B cells; BANK1 and MS4A1 for DC cells; KIT for mast cells; MKI67 for stem cells.

Analysis of Bulk Gene Data

The bulk gene expression and patient information of 722 cases (including 545 endometrial cancer tissues and 177 normal endometrium samples) were downloaded from The Cancer Genome Atlas (TCGA) databases. The comparison of gene expressions between endometrial cancer group and normal group was performed.

Cell-Cell Communication Analysis

Cell-cell communication analysis was conducted using the CellChat package (version 1.6.1). CellChat objects were generated from the Seurat data after cell annotation, and ligand-receptor interactions were identified to infer potential signaling pathways among different cell populations. The inferred interactions were then integrated with a protein-protein interaction network to characterize communication patterns. In addition, we specifically assessed the CXCL signalling pathway intercellular communication between each cell type to uncover potential functional crosstalk.

Statistical Analysis

Statistical analyses were performed using R software (version 4.4.0). For single-cell RNA sequencing data, comparisons of gene expression between two groups were conducted using the Wilcoxon rank-sum test, while comparisons among more than two groups were performed using the Kruskal–Wallis test. For bulk RNA-seq data obtained from TCGA, two-group comparisons were carried out using either Student's *t*-test or the Wilcoxon test, as appropriate. Data distribution was not assumed to be normal for single-cell expression analyses, and non-parametric tests were therefore primarily applied. Given the exploratory and hypothesis-generating nature of this study, statistical analyses focused on predefined genes and signaling pathways of interest, including CXCL family members and ACKR1, and formal multiple testing correction was not applied. A two-sided *P* value < 0.05 was considered statistically significant. Statistical results are presented using standard significance notation (*P* < 0.05, **P* < 0.01, ***P* < 0.001).

Results

Single-Cell Transcriptomic Clustering

We performed integrative clustering analysis using matrix data from 10 endometrial carcinoma tissues and 8 control tissues, which resulted in the identification of 30 subclusters (clusters 0–29) (Figure 1A). Figure 1B showed the tissue origins of these single-cell subclusters across the 18 samples, revealing that the 18 samples were relatively evenly distributed among the 30 subclusters. This indicated that cells from different samples were relatively evenly distributed across clusters, suggesting that major batch-driven clustering patterns were not observed.

Cellular Composition of the Tumor Microenvironment in Endometrial Cancer

Based on the cell marker database CellMarker 2.0 and machine-assisted annotation, we clustered the scRNA-seq of 18 samples into 30 distinct cellular subpopulations (Figure 2). Subclusters 7, 9, 16, and 18 were defined as epithelial cells; subclusters 1, 5, 11, 14, 15, 19, 20, 24, 27, and 29 as stromal cells; subclusters 0, 2, 10, 12, and 28 as T cells; subclusters 3 and 13 as perivascular cells; and subcluster 4 as endothelial cells. In addition, subcluster 6 corresponded to NK cells, subcluster 8 to macrophages, subcluster 17 to NKT cells, subcluster 21 to B cells, subcluster 22 to stem cells, subcluster 23 to dendritic cells, and subclusters 25 and 26 to mast cells. Therefore, the tumor microenvironment of endometrial carcinoma comprised of epithelial cells, stromal cells, T cells, perivascular cells, endothelial cells, NK cells, macrophages, NKT cells, B cells, stem cells, dendritic cells and mast cells.

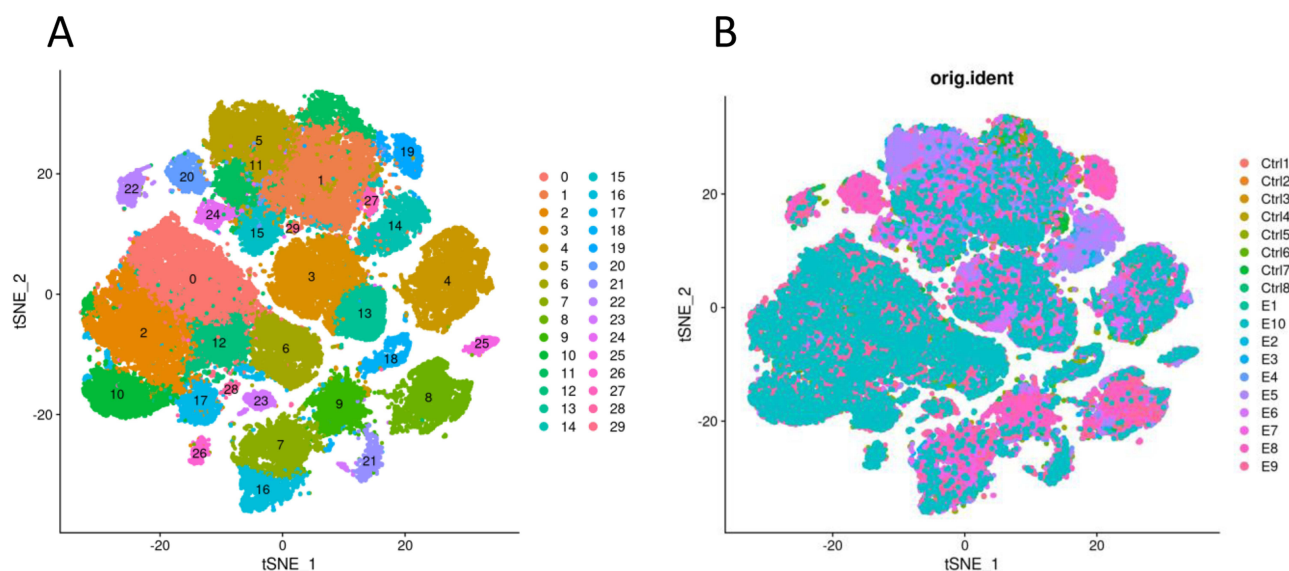


Figure 1 Single-cell transcriptomic clustering plot of endometrial cancer. **(A)** tSNE plot of cell subpopulations, showing that 30 cell clusters of 10 endometrial carcinoma tissues and 8 control tissues. **(B)** Sample origin plot of cell clusters, exhibiting a relatively uniform distribution of subpopulations across the 18 tissue samples.

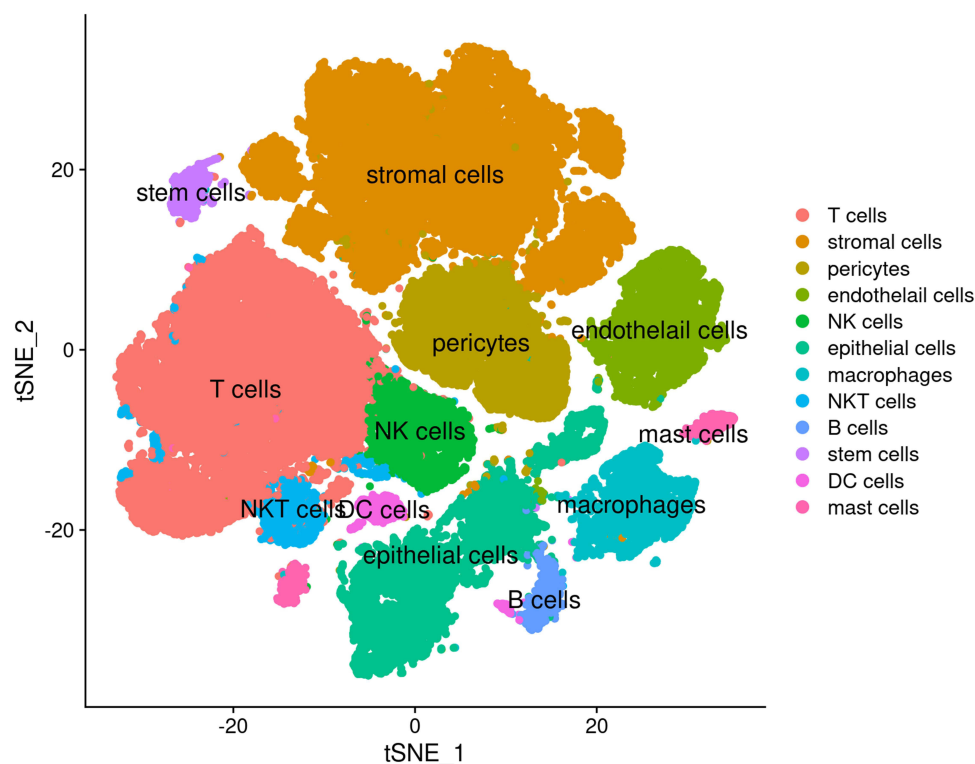


Figure 2 Cell type map of endometrial cancer based on scRNA-seq. After defining the 30 cell clusters, the 18 tissue samples were composed of epithelial cells, stromal cells, T cells, perivascular cells, endothelial cells, NK cells, macrophages, NKT cells, B cells, stem cells, DCs and mast cells.

The CXCL Signaling Pathway in Endometrial Cancer

In the above analysis, we identified the cellular composition of the tumor microenvironment in endometrial cancer. The cell–cell interaction network illustrated both the number (Figure 3A) and strength (Figure 3B) of intercellular communications among different cell types, indicating extensive yet heterogeneous interactions among epithelial cells, stromal cells, T cells, pericytes, endothelial cells, NK cells, macrophages, NKT cells, B cells, stem cells, dendritic cells, and mast cells. The CXCL signaling

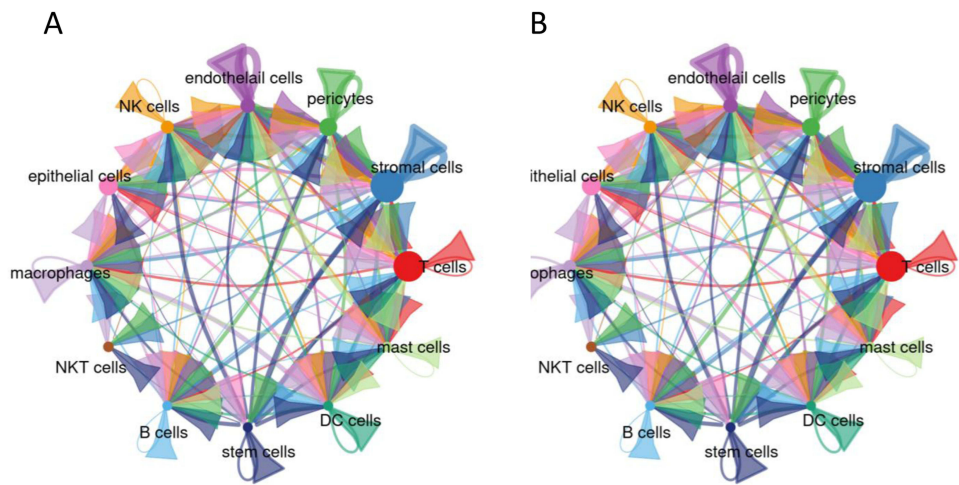


Figure 3 Cell-cell interaction plot. **(A)** Cell interaction number plot, showing the number of cell-cell communications among different cell types. **(B)** Cell interaction strength plot, exhibiting the intensity of cell-cell communications among different cell types. Note: interaction number, count of significant ligand–receptor pairs inferred between sender→receiver cell groups; interaction strength, sum (or aggregate) of CellChat communication probabilities across all significant ligand–receptor pairs for a sender→receiver edge; edge scaling, edge width is scaled to the interaction number/strength (min–max scaled within this figure/panel); node size, node size proportional to the number of cells in each group.

pathway plays an important role in various malignancies, including endometrial cancer. Analysis of the CXCL signaling pathway identified several dominant ligand-receptor interactions, including CXCL8-ACKR1, CXCL2-ACKR1, CXCL16-CXCR6, and CXCL3-ACKR1 (Figure 4A). Among these interactions, the CXCL8-ACKR1 axis exhibited the strongest signaling contribution. Furthermore, tSNE visualization revealed that ACKR1 expression was primarily enriched in endothelial cells (Figure 4B), suggesting that CXCL-mediated signaling may regulate endothelial cell function and angiogenesis in the tumor microenvironment.

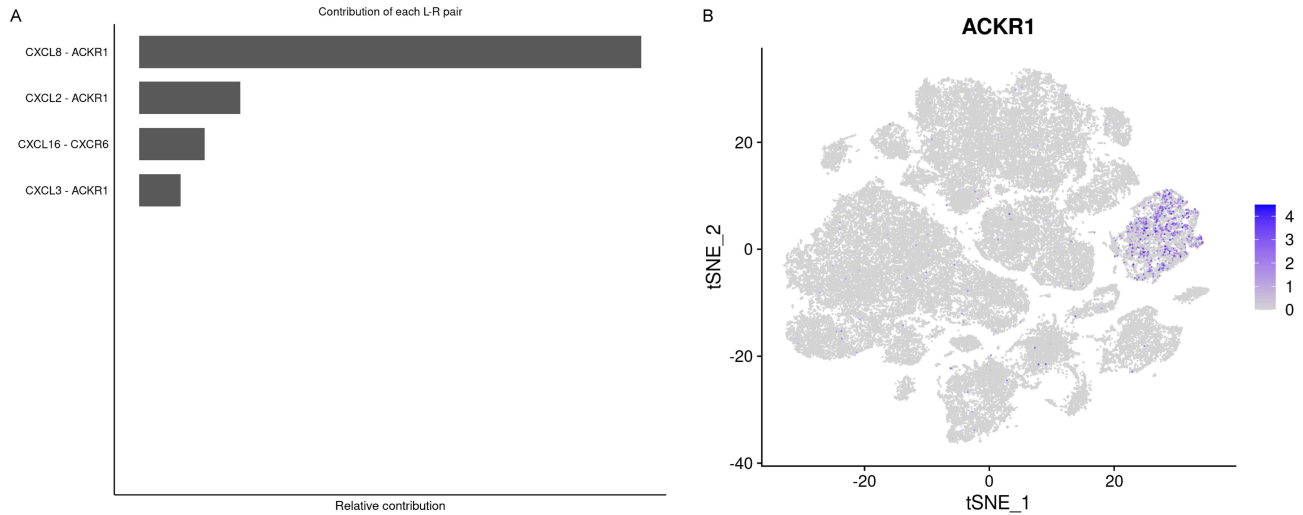


Figure 4 CXCL signaling pathway diagram and cellular distribution of ACKR1 in endometrial cancer. **(A)** Relative contribution of major ligand–receptor interactions involved in the CXCL signaling pathway, as inferred from cell–cell communication analysis. The CXCL8-ACKR1 axis showed the strongest signaling contribution, followed by CXCL2-ACKR1, CXCL16-CXCR6, and CXCL3-ACKR1. **(B)** tSNE visualization of single-cell transcriptomic data showing the expression distribution of ACKR1 across cell populations. ACKR1 expression is predominantly enriched in endothelial cells.

CXCL8/2/3 Were Over-Expressed in Epithelial Cells, Stromal Cells and Tumor Tissues of Endometrial Carcinoma

To assess the expression patterns of CXCL8/2/3 in epithelial cells, violin plots were generated to compare their levels between endometrial carcinoma and normal control tissues. As shown in Figure 5A, CXCL8/2/3 were markedly upregulated in epithelial cells of endometrial carcinoma. Similarly, analysis of stromal cells revealed significantly increased expression of CXCL8/2/3 in tumor-associated stroma compared with normal counterparts (Figure 5B). Furthermore, Figure 5C demonstrated that CXCL8/2/3 expression was globally elevated in endometrial carcinoma tissues relative to normal endometrium, indicating that their overexpression may contribute critically to the development and progression of endometrial carcinoma.

CXCL-ACKR1 May Mediate Angiogenesis in Endometrial Cancer

We further mapped the CXCL receptor-ligand network in endometrial cancer (Figure 6). It was observed that CXCL8-ACKR1, CXCL2-ACKR1, and CXCL3-ACKR1 acted on endothelial cells. Given the high expression of CXCL8/2/3 in epithelial cells, stromal cells, and tumor tissues of endometrial cancer, it suggested that epithelial and stromal cells in the tumor could secrete CXCL8/2/3. These chemokines acted on surrounding endothelial cells through ACKR1, thereby mediating angiogenesis in endometrial cancer and promoting tumor progression. Therefore, CXCL-ACKR1 played

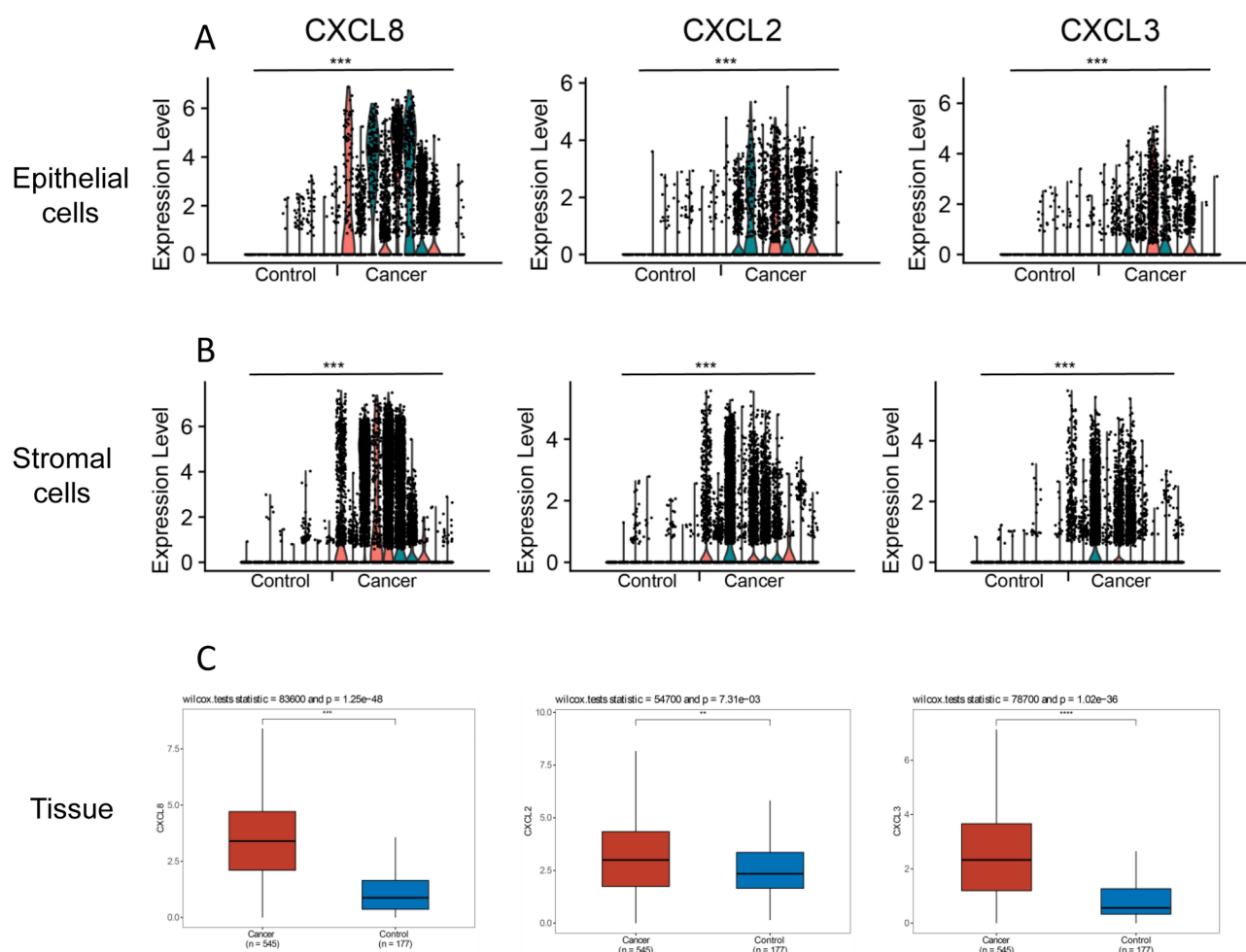


Figure 5 CXCL8/2/3 expression in epithelial cells, stromal cells, and tissues. (A and B) The violin plot showed that the expressions of CXCL8/2/3 were increased in both epithelial and stromal cells of endometrial cancer compared to these of normal endometrial tissue. (C) The box plot showed that CXCL8/2/3 were over-expressed in endometrial cancer tissues when compared to normal control endometrium. ** $P < 0.01$, and *** $P < 0.001$.

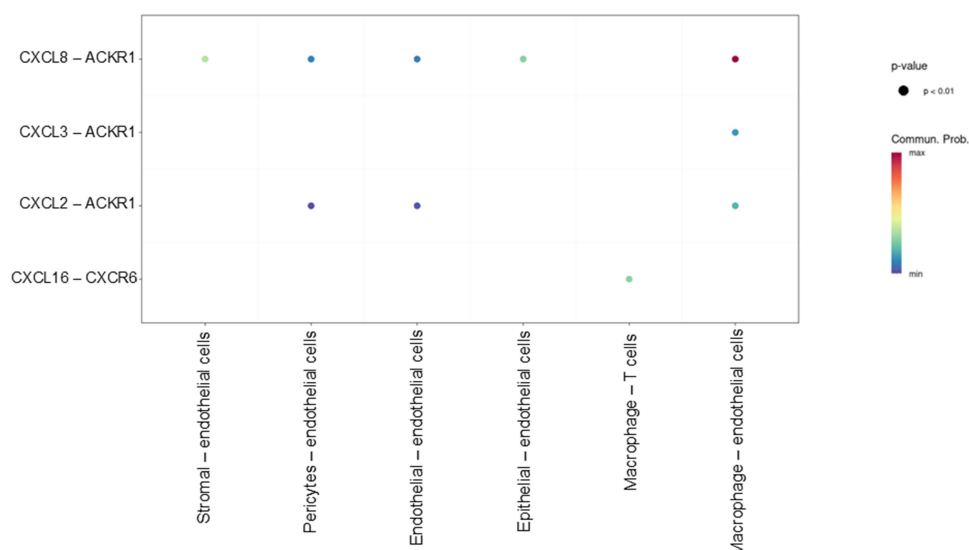


Figure 6 CXCL ligand-receptor map in endometrial cancer. CXCL8-ACKR1, CXCL2-ACKR1 and CXCL3-ACKR1 acted on vascular endothelial cells of endometrial cancer.

a critical role in angiogenesis in endometrial cancer, and targeting ACKR1 could inhibit angiogenesis in endometrial cancer.

Discussion

Angiogenesis plays a crucial role in cancer, acting as a key process in tumor growth and metastasis. Tumors initially rely on host blood vessels for oxygen and nutrients during early growth, but once a certain size is reached, they induce angiogenesis to meet their metabolic demands.¹⁴ This process is driven by various pro-angiogenic factors, such as Vascular Endothelial Growth Factor (VEGF), which promote the proliferation and migration of endothelial cells, forming immature and structurally abnormal tumor blood vessel networks.¹⁵ These vessels not only supply nutrients to the tumor but also serve as channels for cancer cells to enter the bloodstream and metastasize to distant organs.¹⁶ Angiogenesis also plays a critical role in the occurrence and progression of endometrial cancer. However, tumor-associated blood vessels often exhibit structural abnormalities and functional deficiencies, leading to tumor hypoxia in the microenvironment, which stimulates the release of additional pro-angiogenic factors, creating a vicious cycle. Therefore, angiogenesis is not only an important marker of endometrial cancer biological behavior but also a significant target for prognostic evaluation and therapeutic intervention.¹⁷

In endometrial cancer tissues, the expression of pro-angiogenic factors like VEGF is significantly elevated, promoting the formation of new blood vessels and accelerating tumor progression. However, VEGF-targeted drugs, such as bevacizumab, often do not achieve ideal therapeutic effects in clinical practice, indicating that other factors may also play important roles in angiogenesis.¹⁸ Recent studies have found that the failure of anti-VEGF treatment is often accompanied by the activation of other angiogenic pathways, such as endothelial-like transformation of tumor cells, vasculogenic mimicry (VM),¹⁹ and the secretion of additional pro-angiogenic factors by tumor stromal cells, collectively contributing to tumor escape from VEGF inhibition.²⁰ Research has shown that the CXCL family plays an important role in tumor angiogenesis and progression,^{21,22} but studies in endometrial cancer are limited. Therefore, we used scRNA-seq sequencing to investigate the CXCL signaling pathways in endometrial cancer.

CXCL signaling has been shown to contribute to endothelial cell activation and the formation of tumor vasculature in various cancers. However, the role of CXCL-ACKR1 signaling in endometrial cancer is less understood. Our study, by leveraging single-cell RNA sequencing and cell-cell communication analysis, provides one of the first comprehensive insights into the CXCL-ACKR1 axis within the endometrial cancer microenvironment, highlighting its potential importance in angiogenesis specific to this malignancy. Notably, the interaction between CXCL chemokines and ACKR1 on endothelial cells in the context of endometrial cancer may be influenced by unique factors such as estrogen

and progesterone signaling, which are less prominent in other cancer types. These findings suggest that the CXCL–ACKR1 axis could offer a novel therapeutic target for anti-angiogenic strategies in endometrial cancer, an area where effective therapies are still lacking.

The emergence of scRNA-seq has opened new directions for tumor research. Compared to traditional bulk transcriptome analysis, scRNA-seq can reveal gene expression differences between cells at a single-cell resolution, effectively addressing the high heterogeneity of tumors.²³ Research on the tumor microenvironment is also significant, as it can precisely characterize the composition and functional states of immune cells, fibroblasts, and endothelial cells, clarifying their key roles in immune evasion and angiogenesis.²⁴ scRNA-seq can also uncover internal signaling pathways in tumors, providing a theoretical basis for precision medicine and the development of novel targeted strategies.²⁵ In this study, we integrated single-cell data from multiple endometrial cancer samples and performed single-cell level pathway analysis, identifying CXCL8–ACKR1, CXCL2–ACKR1, CXCL16–CXCR6, and CXCL3–ACKR1 as the main CXCL signaling pathways. These results suggest that CXCL-mediated ACKR1 plays an important role in endometrial cancer. We found that ACKR1 is primarily expressed on endothelial cells, indicating its potential involvement in angiogenesis in endometrial cancer. Single-cell data and databases both show that, compared to normal endometrial tissue, the expression of CXCL8/2/3 is increased in endometrial cancer tissues. By acting on ACKR1 on endothelial cells, these factors mediate angiogenesis in endometrial cancer. Therefore, the CXCL–ACKR1 signaling axis may represent a potential regulator of angiogenesis in endometrial cancer and could serve as a promising candidate for further functional investigation. These findings are consistent with emerging evidence highlighting the importance of cancer biomarkers and targeted therapy in tumor progression and early diagnosis, as well as the growing role of precision medicine in oncology.^{26–29}

Several limitations of this study should be acknowledged. This work is primarily hypothesis-generating. Although we identified a CXCL8/2/3–ACKR1 communication axis and tumor-associated expression changes consistent with endothelial-targeted signaling, we did not directly quantify endothelial angiogenesis gene programs or endothelial state transitions, nor did we perform experimental validation. Future studies incorporating angiogenesis pathway scoring, endothelial phenotyping, and functional assays will be needed to confirm whether CXCL–ACKR1 signaling causally promotes angiogenesis in endometrial cancer.

Conclusion

This study provides an integrative single-cell transcriptomic analysis of the angiogenic microenvironment in endometrial cancer and highlights a potential association between CXCL chemokines and ACKR1-expressing endothelial cells. Through cell-type-resolved expression profiling and ligand–receptor interaction inference, our findings suggest that the CXCL–ACKR1 signaling axis may be involved in tumor-associated angiogenic regulation. Importantly, these observations are hypothesis-generating and based on computational inference rather than direct functional validation. Further experimental and clinical studies will be required to elucidate the mechanistic role of CXCL–ACKR1 signaling and to determine its potential relevance as a therapeutic target in endometrial cancer. Together, this work provides a transcriptomic framework that may help guide future investigations into angiogenesis-related intercellular communication in endometrial cancer.

Abbreviations

TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; PCA, principal component analysis; VEGF, Vascular Endothelial Growth Factor; VM, vasculogenic mimicry.

Ethics Approval

All datasets used in this study were obtained from publicly available databases (GEO and TCGA), and no identifiable personal information was involved. According to the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (issued February 18, 2023, China), ethical review may be exempted under Article 32 for studies that (1) use legally obtained, publicly available data or biological materials, or (2) involve anonymized data where

individuals cannot be identified and no harm to subjects is expected. Therefore, this study is exempt from ethical review in accordance with the above national legislation.

Disclosure

The author(s) report no conflicts of interest in this work.

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