

Single-cell and spatial transcriptomics reveal that the CXCL12–CXCR4 axis drives the immune-desert phenotype in small cell lung cancer by recruiting immunosuppressive CXCR4⁺ neutrophils and S100A8⁺ monocytes

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ABSTRACT

Background Small cell lung cancer (SCLC) is a recalcitrant malignancy with limited responses to immunotherapy, largely due to its uniquely immunosuppressive tumor microenvironment (TME). However, the molecular mechanisms driving this phenotype remain incompletely understood.

Methods We integrated single-cell RNA sequencing and Xenium in situ spatial transcriptomics to analyze the immune microenvironment of five SCLC and four non-small cell lung cancer (NSCLC) samples. Multiplex immunofluorescence was used to validate cell types and gene expression in the same tissue specimens, and animal models were employed to verify the key mechanistic pathway.

Results SCLC displayed a distinct immune landscape compared with NSCLC, with increased infiltration of C-X-C motif chemokine receptor 4 (CXCR4)⁺ neutrophils (via neutrophil extracellular traps) and S100A8⁺ monocytes (toward an M2-like phenotype), and reduced CD8⁺ T-cell infiltration. Malignant epithelial cells in SCLC highly expressed CXCR4, regulated by transcription factors ISL LIM homeobox 1 and distal-less homeobox 5, which promoted immunosuppression. The C-X-C motif chemokine ligand 12 (CXCL12)–CXCR4 axis mediated competitive inhibition, impairing T-cell recruitment while enhancing neutrophil accumulation. Monocytes in SCLC shifted toward an M2-like phenotype, weakening antigen presentation. Xenium spatial transcriptomics confirmed colocalization of CXCR4⁺ neutrophils and S100A8⁺ monocytes with tumor cells at the tumor-normal interface, while CD8⁺ T cells were spatially segregated. In vivo experiments showed that CXCR4 inhibition reduced SCLC tumor growth, decreased immunosuppressive cell infiltration, and enhanced CD8⁺ T-cell accumulation.

Conclusions The CXCL12–CXCR4 axis, together with immunosuppressive CXCR4⁺ neutrophils and S100A8⁺ monocytes, is a key driver of the immune-desert phenotype in SCLC. Targeting this axis holds promise as a therapeutic strategy to remodel the immunosuppressive TME and improve the efficacy of immunotherapy for SCLC.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Small cell lung cancer (SCLC) has a highly immunosuppressive “immune-desert” tumor microenvironment (TME) and poor immunotherapy response.
- ⇒ Immune checkpoint inhibitors (ICIs) show limited efficacy, but the spatial/cellular mechanisms driving the immune-desert phenotype remain unclear, hindering targeted therapy development.

WHAT THIS STUDY ADDS

- ⇒ We delineate a mechanism by which the C-X-C motif chemokine ligand 12 (CXCL12)–C-X-C motif chemokine receptor 4 (CXCR4) axis drives the SCLC immune desert.
- ⇒ First, ISL LIM homeobox 1/distal-less homeobox 5-driven tumor cells secrete CXCL12.
- ⇒ Second, this recruits immunosuppressive CXCR4⁺ neutrophils and S100A8⁺ M2-like monocytes, leading to CD8⁺ T-cell exclusion and impaired major histocompatibility complex-I expression.
- ⇒ Finally, CXCR4 inhibition therapeutically reverses these effects, curtailing tumor growth and enhancing antitumor immunity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ This work provides a high-resolution SCLC TME map to guide CXCL12–CXCR4 research.
- ⇒ Clinically, CXCR4 inhibitors may remodel TME and overcome ICI resistance.
- ⇒ Practically, single-cell RNA sequencing/Xenium-based stratification can identify patients who benefit from CXCR4-targeted therapies, reducing ineffective treatment and advancing personalized care.

INTRODUCTION

Lung cancer (LC) has the highest global incidence and mortality.¹ Small cell lung cancer

(SCLC) accounts for only approximately 15%–20% of all cases but is highly invasive with poor prognosis.² Although the introduction of immune checkpoint inhibitors (ICIs) has improved therapeutic outcomes to some extent, the overall response rate remains relatively low.³ Therefore, comprehensive research on the pathogenesis of SCLC and its complex immune microenvironment is urgently needed.

However, the molecular regulatory network of SCLC is highly complex, involving dynamic imbalances in gene expression, dysregulation of epigenetic modifications, cross-talk among multiple signaling pathways, and intricate interactions between immunosuppressive cells and tumor cells in the tumor microenvironment (TME), leading to limitations in subtyping accuracy and interpatient variability in treatment responses.⁴ This complexity makes it challenging for analyses solely based on population-level molecular characteristics to comprehensively explain the mechanisms underlying SCLC initiation and progression and further complicates the accurate identification of potential therapeutic targets and drug resistance-related molecular events.

Although ICIs have been incorporated into chemotherapy regimens in recent years, their clinical benefits remain limited.⁵ From the perspective of the immune microenvironment, SCLC typically exhibits an immune-desert phenotype, characterized by extremely low infiltration of immune cells in the TME, low expression of immune checkpoint molecules, and frequent enrichment of fibrotic stroma and immunosuppressive cells, which together form a strong immunosuppressive barrier.⁶ In contrast, non-small cell lung cancer (NSCLC) frequently displays an immune-infiltrated phenotype, with infiltration of T and B cells and activation of immune checkpoint molecules in tumor tissues, suggesting active immune responses.⁷ This distinction directly contributes to markedly different responses to immunotherapy between these two types of LC. The formation of the immune desert in SCLC may be related to the recruitment of immunosuppressive cells via chemokines secreted by tumor cells, whereas in the immune-infiltrated subtypes of NSCLC, immune escape more commonly relies on the activation of the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway or T-cell exhaustion. Therefore, comparative studies on these two immune microenvironment subtypes may help reveal the specific mechanisms of immune escape in SCLC.

The emergence of single-cell RNA sequencing (scRNA-seq) technology has provided a powerful approach to address these challenges. This method not only enables the resolution of transcriptomic profiles of immune, stromal, and tumor cells within the TME at single-cell resolution but also accurately identifies rare cell subpopulations that are undetectable by traditional bulk sequencing.⁸ As an in situ spatial transcriptomic platform, Xenium enables subcellular, high-resolution spatial localization of hundreds of RNAs in intact tissues, allowing three-dimensional integrated analysis

of “cellular phenotype–molecular characteristics–spatial location”.⁹ The integration of scRNA-seq and Xenium not only precisely deciphers the gene expression profiles of individual cells but also defines their spatial context within the tissue. This combined approach holds promise for constructing a high-resolution single-cell atlas of the SCLC immune microenvironment, elucidating the cellular basis of immune desert formation, uncovering spatial interaction patterns between tumor cells and immune cell subsets, and identifying key signaling hubs that drive the development of an immunosuppressive microenvironment.

Therefore, this study systematically dissected SCLC and NSCLC molecular characteristics via scRNA-seq/Xenium, characterized immune cell transcriptomes, validated spatial distribution via multiplex immunofluorescence (mIF), and verified key pathways in animal models to elucidate SCLC’s immune microenvironment and identify therapeutic targets.

RESULTS

Global immune landscape in SCLC and NSCLC

To comprehensively evaluate the immune landscape of SCLC, we collected nine tumor samples (five SCLC, four NSCLC) and performed scRNA-seq and in situ spatial transcriptomic analysis (figure 1A). After stringent filtering (online supplemental Tables S1 and S2, Figure S1A–H), 47,761 cells were obtained (24,133 NSCLC, 23,628 SCLC; figure 1B), with 8 major cell types identified via marker genes: B cells (CD79A, JCHAIN), endothelial cells (CLDN5, CDH5), epithelial cells (EPCAM, KRT18), fibroblasts (DCN, PDGFRA), mast cells (CPA3, KIT), monocytes (LYZ, C1QA), neutrophils (CSF3R, FCGR3B), and T/NK cells (CD3D, NCAM1) (figure 1C). Cell cluster proportions were visualized (figure 1D, S1I). χ^2 tests (figure 1E) and differential gene volcano plots (online supplemental Figure S1J) revealed significant differences in cell type composition between SCLC and NSCLC, with SCLC showing >twofold higher neutrophil proportion (online supplemental Figure S1K). Subset/marker expression was verified by scRNA-seq (online supplemental Figure S1L), and spatial distribution in tissue sections was examined via Xenium technology (figure 1F, online supplemental Figure S2A and B). Integrating Xenium and scRNA-seq data, we identified overlapping expressed genes, performed Pearson correlation analysis, and constructed heatmaps (figure 1G, online supplemental Figure S2C). Results showed significant consistency in cell type definition between the two methods, confirming Xenium’s reliability for studying LC cell composition and immune microenvironment.

Differences in epithelial cells between NSCLC and SCLC result in distinct immune microenvironments

From LC samples, 16,779 epithelial cells were isolated and divided into three subsets (figure 2A). Using stromal/macrophage cells as references, inferCNV analysis

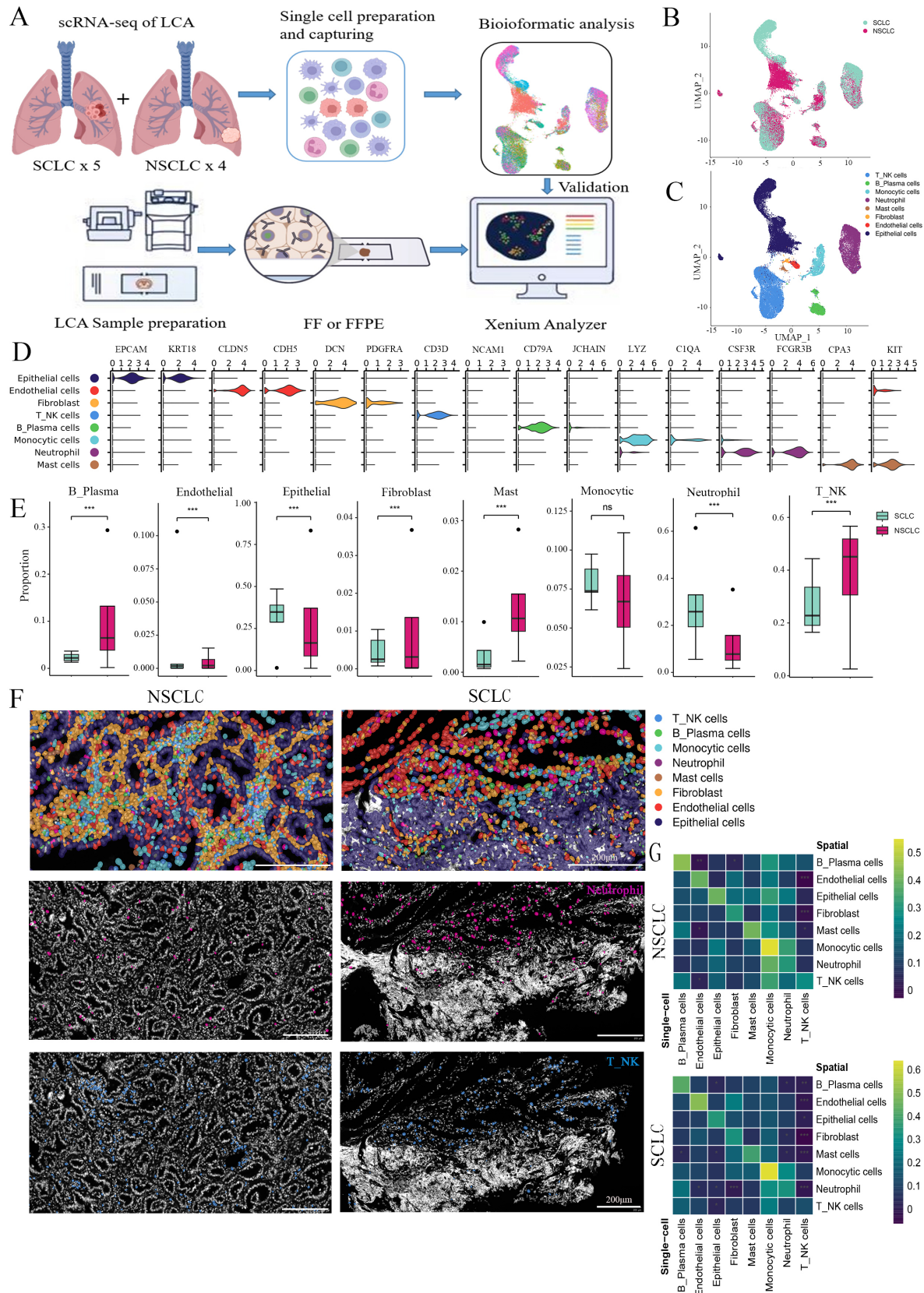


Figure 1 Global immune landscape in SCLC and NSCLC. (A) Schematic representation of the experimental design and analysis workflow. (B) UMAP plot showing sample groupings. (C) UMAP plot of eight cell types. (D) Violin plots of marker genes for the eight cell clusters. (E) Box plot of cell proportions. (F) Xenium in situ spatial transcriptomic staining of cell subpopulations. (G) Pearson correlation heatmap comparing scRNA-seq and Xenium in situ spatial transcriptome analyses. FF, fresh frozen; FFPB, formalin-fixed, paraffin-embedded; LCA, lung cancer; NK, natural killer; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; scRNA-seq, single-cell RNA sequencing; UMAP, Uniform Manifold Approximation and Projection.

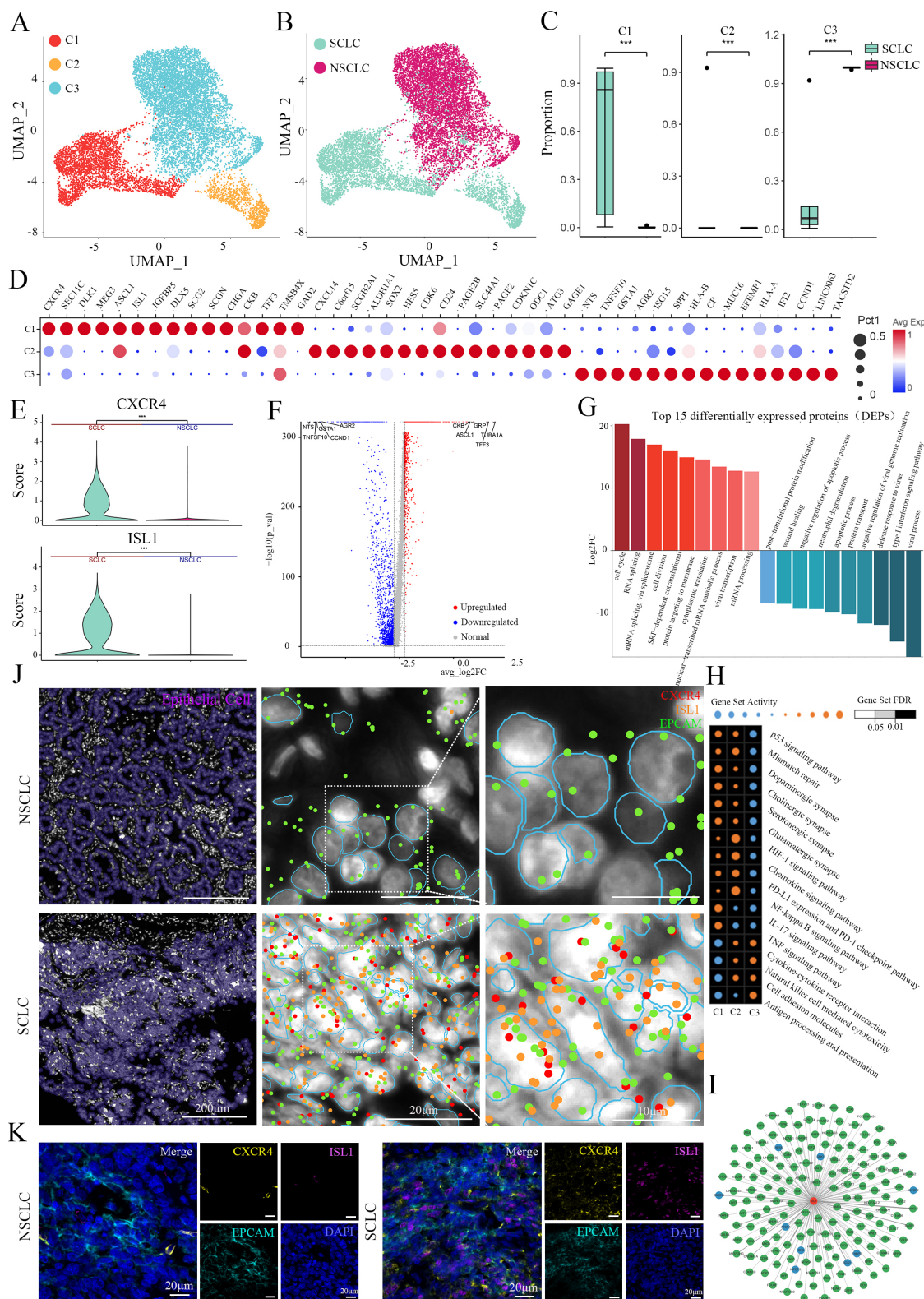


Figure 2 Proportion and distribution of CXCR4⁺ epithelial cells. (A) UMAP clustering of malignant cells. (B) UMAP plot of SCLC and NSCLC groups. (C) Box plot comparing cell proportions between SCLC and NSCLC groups. (D) Bubble plot of marker genes in C1, C2, and C3. (E) Violin plots showing CXCR4 and ISL1 expression in the two groups. (F) Volcano plot of differentially expressed genes between SCLC and NSCLC. (G) Bar plot of the top 15 differentially expressed proteins between SCLC and NSCLC. (H) Quantitative set analysis for gene expression pathway analysis in SCLC and NSCLC. (I) Ball-and-stick diagram showing transcription factors and their target genes. (J) Distribution and expression of CXCR4⁺ and ISL1⁺ epithelial cells detected using Xenium spatial transcriptomic analysis. (K) Immunofluorescence staining of CXCR4⁺ and ISL1⁺ epithelial cells. CXCR4, C-X-C motif chemokine receptor 4; FC, fold change; FDR, false discovery rate; ISL1, ISL LIM homeobox 1; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UMAP, Uniform Manifold Approximation and Projection.

classified cells with mean square >0.05 and correlation coefficient >0.5 as malignant, identifying 12,628 malignant cells (figure 2B, online supplemental Figure S3A-C). Proportion statistics (online supplemental Figure S3D) and χ^2 tests (figure 2C) revealed differential distribution of the three malignant subsets between SCLC and NSCLC: C1 (predominantly SCLC), C2 (SCLC-2 specific), and C3 (predominantly NSCLC). Signature gene analysis showed C1 enriched for C-X-C motif chemokine receptor 4 (*CXCR4*) and ISL LIM homeobox 1 (*ISL1*), C2 for CDK6/HES5, and C3 for human leukocyte antigen (*HLA*)-A, *HLA*-B, and interferon-stimulated gene 15 (*ISG15*) (figure 2D). In addition, SCLC also expressed *ASCL1*, *CD24*, *CKB*, *CHGA*, *CXCR4*, *TFF3*, *ISL1*, and distal-less homeobox 5 (*DLX5*) (online supplemental Figure S3E). Between SCLC and NSCLC, transcription factors *ISL1*/*DLX5* were significantly activated in C1, with targets including *CXCR4*, suggesting SCLC may drive immune escape via *ISL1*-mediated *CXCR4* overexpression. Differential gene analysis identified significantly upregulated *CXCR4*/*ISL1* in SCLC (figure 2E). The results were visualized in a volcano plot (figure 2F), highlighting the top 20 upregulated and top 10 downregulated genes (figure 2F). Enrichment analysis showed SCLC upregulated genes linked to proliferation, drug resistance (*CKB*)¹⁰ and endocrine function (*CHGA*)¹¹ while NSCLC upregulated genes related to TME immune regulation (figure 2G). Overlap between subset-specific signature genes and differentially expressed genes further validated subset functions. Quantitative set analysis for gene expression (QuSAGE) pathway analysis showed C1/C2 activated pathways associated with malignant progression (proliferation, mismatch repair, hypoxia), while C3 activated immune pathways (antigen presentation, natural killer (NK) cell cytotoxicity; figure 2H), indicating NSCLC (C3) has stronger immune activity and SCLC (C1/C2) is immune-silent. SCENIC and differential gene analysis generated a ball-and-stick diagram. *ISL1* was highly activated in C1, suggesting a key role in SCLC formation (figure 2I). Xenium spatial transcriptomics (figure 2J, online supplemental Figure S4) and mIF staining (figure 2K) validated *EPCAM*⁺*CXCR4*⁺ cell distribution and expression. Xenium also verified some other key genes (*ASCL1*, *CHGA*, *CXCR4*, *ISL1*; online supplemental Figure S4). In summary, important functional genes such as *CXCR4* and *ASCL1* are regulated by the same transcription factor, *ISL1*, which is a critical regulator of the neuroendocrine characteristics of SCLC,¹² whereas *CXCR4* plays a pivotal role in tumor immune escape.¹³

CXCR4⁺ neutrophils mediate immunosuppression in SCLC

Using scRNA-seq, we identified a substantial number of infiltrating neutrophils in SCLC. These neutrophils expressed classic markers (G0S2, FCGR3B) and high *CXCR4* levels (figure 3A,B, online supplemental Figure S5), leading us to hypothesize their role in competitive immunosuppression—analogue to *CXCR4*⁺ tumor cells that inhibit T-cell infiltration. Further analysis of

differential gene expression (DGE) between SCLC and NSCLC neutrophils revealed alterations in ten core genes. *CD74*: impaired immune recognition¹⁴; *IL1R2*: downregulation reduces inflammatory responsiveness¹⁵; *LGALS3*: alters immune cell function¹⁶; interleukin (IL)-1 receptor-associated kinase 3: disrupts immune signal transduction¹⁷; endoplasmic reticulum aminopeptidase 2: downregulation hinders T-cell recognition¹⁸; *IL-1* receptor antagonist: direct immunosuppression¹⁹; protein tyrosine phosphatase receptor type E: impairs immune activation²⁰; phosphodiesterase 4B: upregulation promotes tumor progression²¹; prostaglandin-endoperoxide synthase 2: upregulation aids angiogenesis/immune evasion²²; matrix metalloproteinase 25: upregulation favors invasion/metastasis (figure 3C).²³ Collectively, these alterations indicate suppression of immune-activating functions, ultimately leading to immune silence.

Gene Ontology (GO)-Biological Process and pathway enrichment analysis showed upregulation of pro-inflammatory chemokines (IL-17, tumor necrosis factor (TNF)). Despite enhanced immune infiltration via strong chemokine binding, *CXCR4* may bind C-X-C motif chemokine ligand 12 (CXCL12) to competitively inhibit immunity, downregulating phagocytosis/antigen presentation. Downregulation of ferroptosis/autophagy pathways weakened tumor cell death mediation (figure 3D). Major histocompatibility complex (MHC) class I molecule expression in neutrophils was significantly reduced in SCLC: violin plots showed lower *HLA*-A/B/C/E/F (figure 3E), and box plot scoring revealed impaired antigen presentation/*CD8*⁺ T-cell activation—indicating immunosuppressive effects. In NSCLC, MHC class I, type II interferons (IFNs), and immune responses correlated positively with *CXCR4*. This correlation was absent in SCLC (figure 3F), confirming SCLC neutrophils' immunosuppressive state. Xenium in situ spatial transcriptomics identified *FCGR3B*⁺ neutrophils, confirming increased *CXCR4*⁺ neutrophil infiltration in SCLC and decreased *IFIT3*⁺/*ISG15*⁺ neutrophils (figure 3G, online supplemental Figure S6). mIF staining further validated these findings (figure 3H).

Distribution and significance of monocytes in SCLC and NSCLC groups

To explore immune microenvironment differences between SCLC and NSCLC, monocytes as a key immune cell subset were investigated. Using scRNA-seq and bioinformatics analysis, we identified four monocyte subtypes from the two groups (figure 4A), with their distribution visualized via Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction (figure 4B). Proportion statistics and χ^2 tests revealed significant inter-group differences: C2 and C3 proportions decreased, while C4 increased in SCLC (figure 4C).

A bubble plot based on marker genes was used to further analyze the functional genes of each cell type. C1: *FOLR2*, highly expressed in specific macrophage subsets,

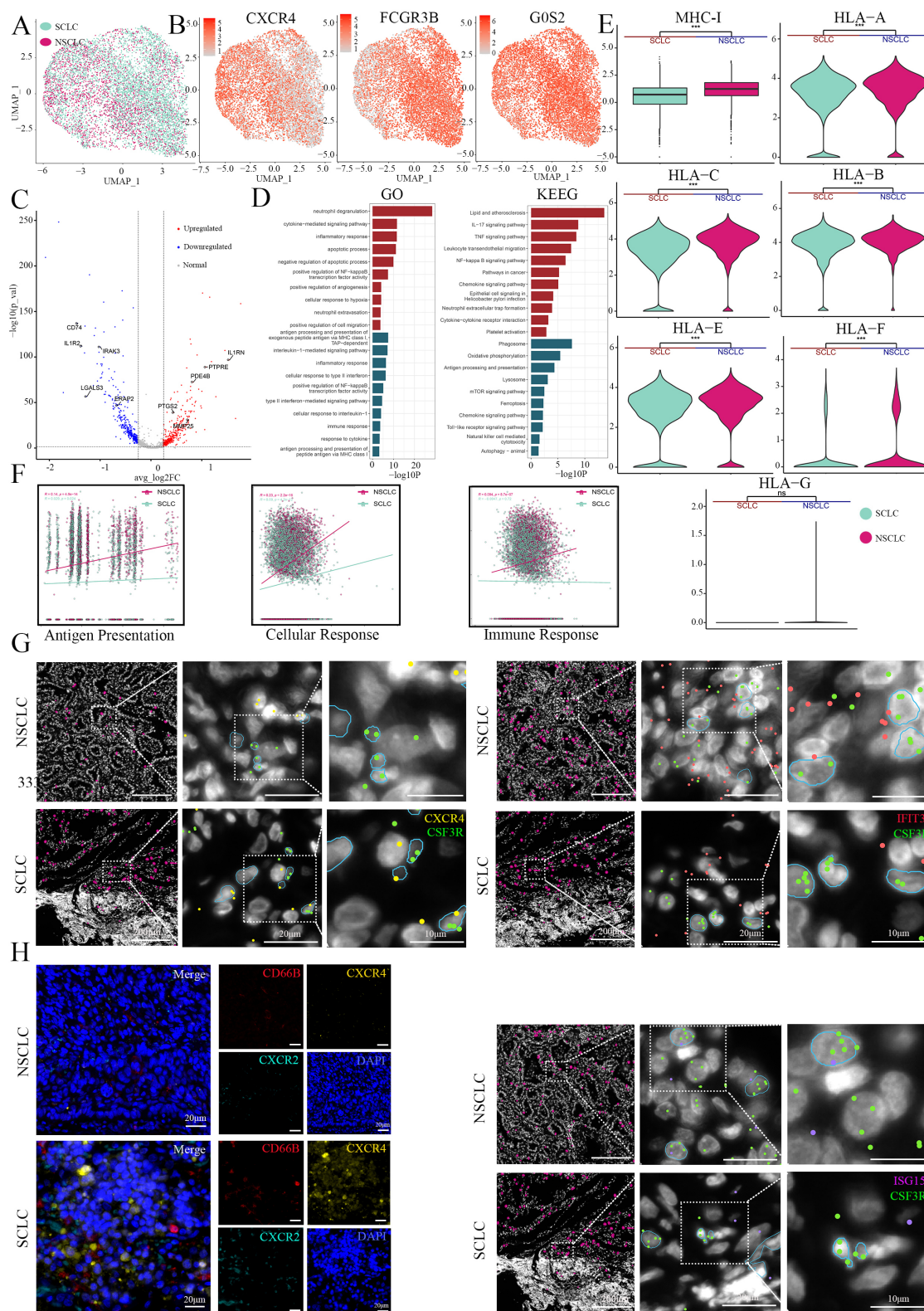


Figure 3 Immunosuppressive effects mediated by *CXCR4*⁺ neutrophils in SCLC. (A) UMAP plot showing sample distribution. (B) UMAP plot of marker genes. (C) Volcano plot of differentially expressed genes. (D) GO-Biological Process and pathway enrichment analysis of differentially expressed genes. (E) Box plot of MHC-I scores and violin plots of associated molecules. (F) Pearson correlation analysis of neutrophil immune functions. (G) Expression and spatial distribution of *CXCR4*, *IFIT3*, and interferon-stimulated gene 15 in *FCGR3B*⁺ neutrophils detected by Xenium in situ spatial transcriptomics. (H) Multiplex immunohistochemistry staining showing *CXCR2* and *CXCR4* expression in *CD66B*⁺ neutrophils. *CXCR4*, C-X-C motif chemokine receptor 4; FC, fold change; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HLA, human leukocyte antigen; MHC, major histocompatibility complex; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UMAP, Uniform Manifold Approximation and Projection.

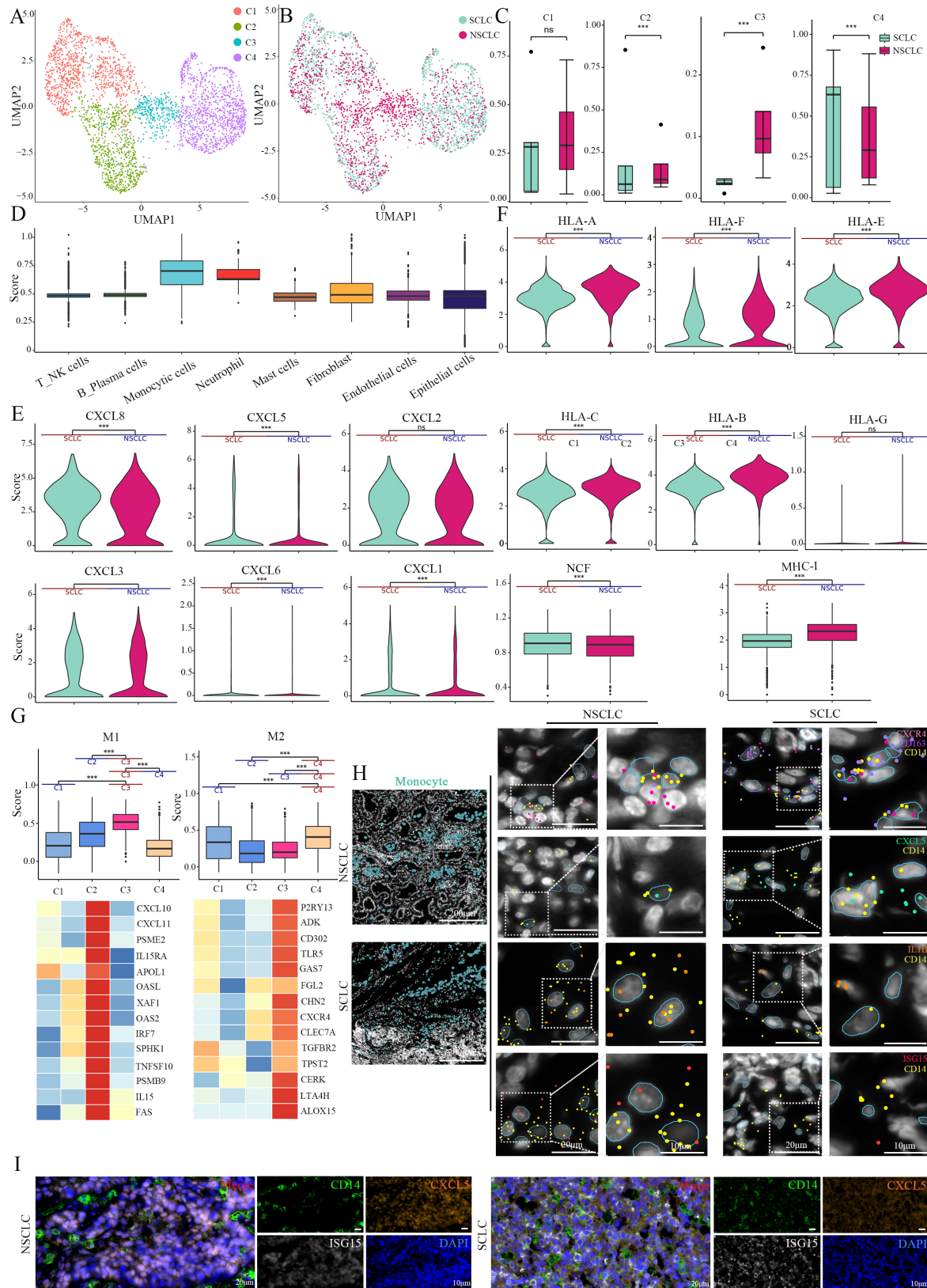


Figure 4 Immunosuppressive effects mediated by monocytic lineage cells in SCLC. (A) UMAP plot of cell types. (B) UMAP plot with group information. (C) Box plot of cell proportions. (D) Box plot of NCF scores across cell clusters. (E) Chemotactic effects of monocytes on neutrophils. (F) Box plot of MHC-I scores and violin plots of related molecules. (G) Box plots (Wilcoxon test) and heatmap of M1 and M2 gene sets. (H) Spatial expression of key monocyte genes in CD14⁺ monocytes. (I) Immunofluorescence staining of key monocyte genes. CXCL, C-X-C motif chemokine ligand; HLA, human leukocyte antigen; MHC, major histocompatibility complex; NCF, neutrophil chemotactic factor; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UMAP, Uniform Manifold Approximation and Projection.

links to macrophage activation and specialized phagocytosis.²⁴ It also expresses MHC class II molecules (HLA-DQA1, HLA-DPB1, HLA-DOA) for antigen presentation, which are critical for immune regulation by presenting antigenic peptides to CD4⁺ T cells and initiating adaptive immunity.²⁵ C2: MMP9⁺ macrophages. The expression of MMP9 suggests that these cells may participate in extracellular matrix remodeling and inflammation regulation. Enriched chemokine-related genes (*CSTB*, *CTSL*, *CCL2*) mediate cell chemotaxis, guiding immune cell directional migration and regulating their distribution in the TME via binding to corresponding receptors. C3: ISG⁺ monocytes. Enriched ISGs highlight their role in antiviral immunity and immune activation signaling.²⁶ IFN-related genes (*IFIT3*, *IFITM3*, *TNFSF10*, *ISG15*) drive immune activation, enhancing host defense via JAK-STAT pathway activation to induce immune cell activation and cytokine secretion.²⁷ C4: S100A8⁺ monocytes. S100A8 associates with TME inflammation and tumor progression.²⁸ S100 family genes (*S100A4*, *S100A8*) interact with intracellular/extracellular targets, activating pathways that promote tumor migration, invasion, and angiogenesis to drive progression. Comparative analysis confirmed C3 (ISG⁺ monocytes) reduction and C4 (S100A8⁺ monocytes) increase in SCLC (online supplemental Figure S7A,B).

Using QuSAGE bubble heatmaps, we systematically analyzed pathway enrichment across cell clusters. C1: Enriched in Th1/Th2 differentiation, phagosome formation, and antigen processing/presentation—key for immune cell activation, immune polarization (Th1/Th2 balance), and adaptive immunity initiation. C2/C3: Enriched in Nuclear Factor-kappa B (NF-κB) signaling, toll-like receptor (TLR) signaling, cytokine-cytokine receptor interactions, and IL-17 signaling. These drive inflammation (NF-κB/TLR) and immune cell recruitment/activation (cytokine interactions, IL-17).²⁹ C4: Enriched in Hypoxia-Inducible Factor 1 (HIF-1) signaling, Vascular Endothelial Growth Factor (VEGF) signaling, and neutrophil extracellular trap (NET) formation, which are critical for tumor hypoxia adaptation, angiogenesis (HIF-1/VEGF), and immune/inflammation regulation (NETs) (online supplemental Figure S7C).³⁰ The major expanded population in SCLC, C4, comprises S100A⁺FCN1⁺ monocytic cells. With weak antigen-presenting/phagocytic functions but activated VEGF/HIF pathways, C4 has strong vascular growth-promoting capacity, supporting its role in SCLC angiogenesis and progression.

Among all cell types, monocytes had the highest neutrophil chemotactic factor (NCF) score, indicating the strongest neutrophil chemotaxis (figure 4D). SCLC exhibited stronger NCF activity, explaining its higher neutrophil infiltration. Violin plots of neutrophil chemokines showed notable changes in CXCL8 and CXCL5. Given CXCL8's excessively high expression risked high background noise in Immunohistochemistry (IHC)/fluorescence staining, CXCL5 was selected

for further analysis to highlight meaningful differences (figure 4E).

Violin plot scoring showed lower MHC-I scores in SCLC (figure 4F), further validated by DGE volcano plots (online supplemental Figure S7D). Diminished MHC-I impairs antigen presentation, weakening CD8⁺ T-cell activation and anti-tumor immunity. Scoring plots and gene set heatmaps showed C3 had the highest M1-type molecule score and strongest M1-gene activation. Its reduction in SCLC reflected decreased M1-polarized macrophages. C4 (CXCR4⁺ immunosuppressive macrophages) increased in SCLC (figure 4G), indicating NSCLC features M1 polarization while SCLC shows M2 polarization. Xenium in situ spatial transcriptomics confirmed *CXCR4*, *CD163*, *CXCL5*, *IL1B*, *ISG15* expression in CD14⁺ monocytes, revealing SCLC monocytes are CXCL5⁺ (neutrophil-recruiting) and M2-polarized (figure 3H, online supplemental Figure S8), further verified by mIF staining (figure 3I).

Competitive immunosuppression leads to impaired infiltration of CD8⁺ T cells in SCLC

CD8⁺ T cells are pivotal for dissecting LC pathogenesis and developing therapies. Distinct immune microenvironments in SCLC and NSCLC drive differences in CD8⁺ T-cell characteristics and functions. Exploring these differences is key to precise LC management. Here, scRNA-seq analysis of 5476 CD8⁺ T cells from LC samples characterized SCLC-specific CD8⁺ T-cell features, highlighting competitive inhibition by tumor cells.

Single-cell clustering classified CD8⁺ T cells into effector memory T cell (Tem), T-cycling, exhausted T cell (Tex), effector T cell (Teff) (Temra), central memory T cell (Tcm), and SCLC/NSCLC-specific subgroups (figure 5A and B). Proportion analysis showed SCLC had increased Tcm/Teff but decreased Tex, Tem, T-cycling (figure 5C, online supplemental Figure S9C). Despite elevated Teff (high GNLY/GZMA) in SCLC, CD8⁺ T cells remained exhausted—likely due to high CXCR4⁺ tumor cells competing for immunoregulatory factors/cytokine binding sites/nutrients, inducing exhaustion and suppressing Teff activation/proliferation/infiltration. Reduced Tem/T-cycling Tex may result from tumor competitive inhibition, while decreased CD8⁺ T-cell infiltration lowers antigen/immunosuppressive factor exposure, contributing to Tex decline.

To further investigate the genetic changes underlying these phenomena, we performed differential expression analysis of the gene expression profiles of CD8⁺ T cells between SCLC and NSCLC (figure 5D–F, S9A). Volcano plots showed SCLC CD8⁺ T cells had upregulated immune checkpoint genes (eg, *HAVCR2*, *TIGIT*)—linked to enhanced exhaustion—and downregulated antigen recognition and signal transduction genes (eg, *CD3*, *CD8*)—indicating impaired antigen presentation (figure 5H).

Gene Set Enrichment Analysis revealed SCLC-enriched CD8⁺ T-cell cytotoxicity/exhaustion gene sets but

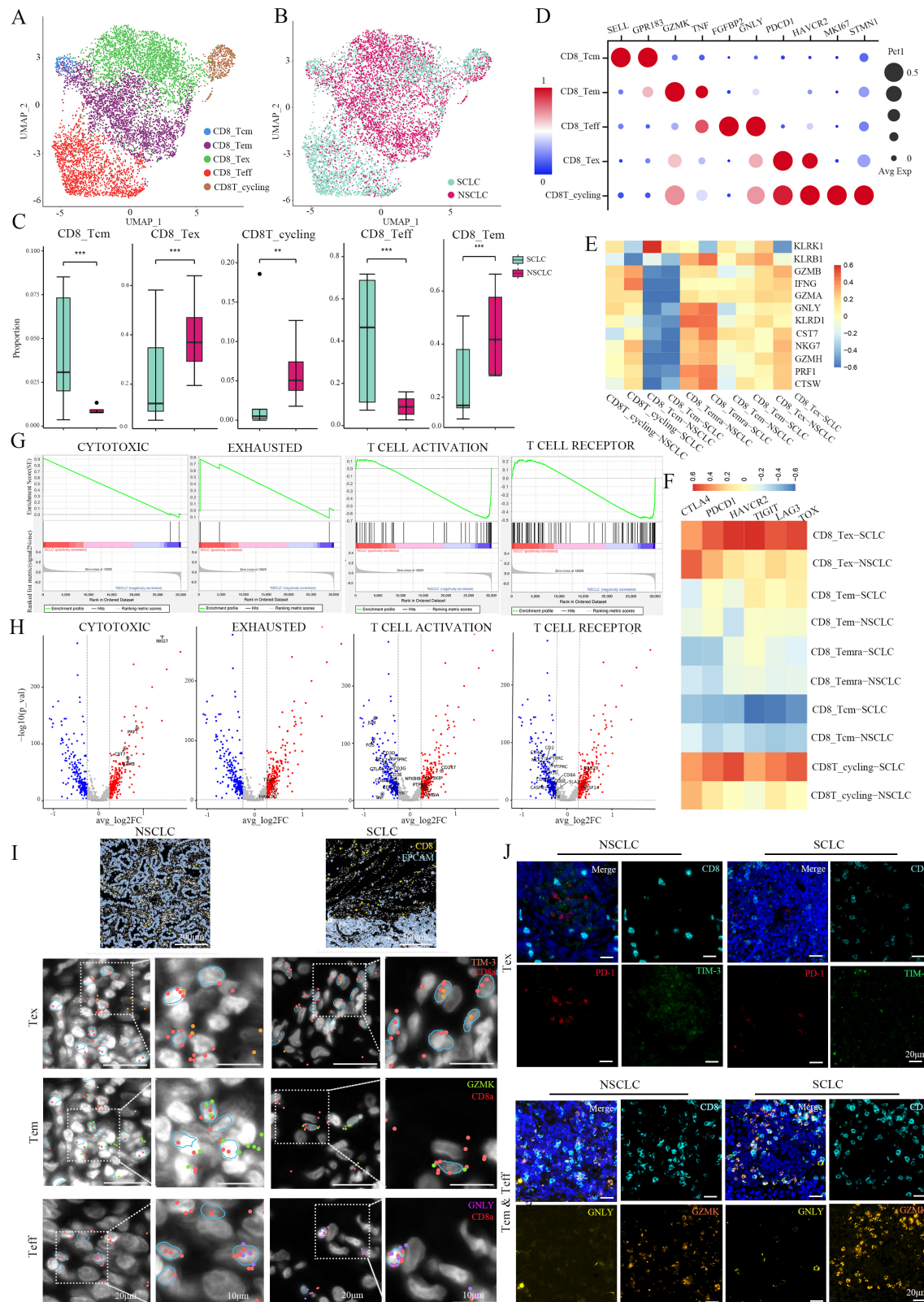


Figure 5 CD8⁺ T-cell subsets and their molecular characteristics in SCLC. (A) UMAP plot of cell types. (B) UMAP plot of groups. (C) Bar chart showing the proportions of CD8⁺ T-cell subsets. (D) Bubble plot of differentially expressed genes in CD8⁺ T-cell subsets. (E) Heatmap of immune checkpoint phenotypes of CD8⁺ T-cell subsets in SCLC and NSCLC. (F) Heatmap of cytotoxic phenotypes in CD8⁺ T subsets from SCLC and NSCLC. (G) Gene Set Enrichment Analysis plots of four key T-cell functions. (H) Volcano plots of four important T-cell functions. (I) Xenium in situ analysis showing the spatial distribution and expression of CD8⁺ T-cell subsets. (J) Multiplex immunofluorescence staining of exhausted and cytotoxic CD8⁺ T cells. FC, fold change; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UMAP, Uniform Manifold Approximation and Projection; Tcm, Central Memory T cell; Teff, Effector T cell; Tem, Effector Memory T cell; Tex, Exhausted T cell.

downregulated T-cell receptor (TCR) signaling/activation sets (figure 5G,H). This may reflect high tumor CXCR4 expression: CXCR4 competes with CD8⁺ TCRs for chemokine binding, disrupting migration/TCR signaling and inhibiting activation. Additionally, tumor competitive inhibition may modulate monocytes via paracrine effects, downregulating MHC-I and further impairing CD8⁺ T-cell tumor recognition/elimination.

Xenium in situ analysis of SCLC CD8⁺ T cells showed fewer T cell-enriched areas than tumor-infiltrated regions (figure 5H, online supplemental Figure S10). T cells rarely entered the tumor core—consistent with ineffective immunity, as Teff failed to penetrate. Tumor cell proliferation and microenvironment remodeling spatially restricted CD8⁺ T-cell migration. High CXCR4 expression reshaped chemokine gradients, disrupting T-cell chemokine sensing/response and hindering tumor migration. mIF staining further validated CD8⁺ T-cell expression/distribution, S, which demonstrated that T cells in SCLC were reduced and exhibited exhaustion (figure 5J).

Activation of the CXCL12–CXCR4 axis promotes SCLC immunosuppressive TME

Cell–cell communication analysis revealed SCLC/NSCLC malignant cell differences (figure 6A): SCLC tumor cells linked to CXCL signaling, while NSCLC tumor cells were not. Further investigation of the key chemokine CXCL12 and its receptor CXCR4 showed NSCLC tumor cells neither produced nor responded to CXCL12, whereas SCLC tumor cells responded to CXCL12 stimulation (figure 6B). This difference is likely the primary driver of SCLC tumor cell competitive inhibition and is closely linked to SCLC metastasis.

Network analysis showed downregulated CXCL/CCL pathways in SCLC—linked to its inhibited T-cell, enhanced neutrophil infiltration. Pathway contribution analysis identified top three SCLC genes: *GALECTIN*, *SPPI*, *CXCL*. Among these, CXCL12/CXCR4 (T-cell infiltration-related) was downregulated (consistent with reduced T cells), CXCL8/CXCR2 (neutrophil-related) upregulated (aligning with increased neutrophils; figure 6C). Ligand-receptor ranking showed SCLC upregulated CXCL8–CXCR2 and downregulated CXCL12–CXCR4, with distinct top pairs in NSCLC (figure 6D).

Spatial distribution analysis showed NSCLC tumor epithelial cells and CD8⁺ T cells were fully intermingled without clear separation, whereas SCLC displayed a distinctly opposite distribution pattern for these two cell types. Similarly, CXCR4⁺ neutrophils showed a similar spatial trend (figure 6E). mIF staining further confirmed these findings (figure 6F). This spatial difference indicates a mutual inhibitory relationship between SCLC tumor and immune cells. CXCL12 stimulation may alter SCLC migration/microenvironment remodeling, spatially restricting immune cells, hindering tumor recognition/cytotoxicity, and forming a tumor-permissive TME.

In vivo validation of the CXCL12–CXCR4 axis in SCLC

To validate CXCL12–CXCR4 axis regulation of SCLC TME, 6–8 weeks old male C57BL/6 mice with subcutaneous tumors received intraperitoneal CXCR4 inhibitor on day 7 (figure 7A). On day 7 post-treatment, SCLC anti-CXCR4 tumors were significantly smaller than controls ($p < 0.0001$), while NSCLC showed no volume difference ($p > 0.05$) (figure 7B)—confirming specific in vivo SCLC growth suppression via CXCR4 inhibition.

To explore the impact of CXCR4 inhibition on the tumor immune microenvironment (TIME), we performed flow cytometry on dissociated tumor tissues. NSCLC had high but unchanged CD8⁺ T-cell proportions with anti-CXCR4 ($p > 0.05$), while SCLC anti-CXCR4 had significantly higher CD8⁺ T cells ($p < 0.001$) (figure 7C,D). CD8⁺PD-1⁺ T cells increased non-significantly in NSCLC ($p > 0.05$) but substantially in SCLC ($p < 0.0001$) (figure 7E,F). In SCLC, the anti-CXCR4 group also showed lower proportions of CD11b⁺Ly6G⁺ neutrophils ($p < 0.0001$) and CXCR4 expression ($p < 0.001$) vs control (figure 7G–J), with mIF confirming these findings (figure 7K,L)—consistent with scRNA-seq data showing enriched CXCR4⁺ neutrophils in SCLC.

Given that CXCL12 is the primary ligand of CXCR4, we measured CXCL12 secretion in tumor tissue supernatants using ELISA. In SCLC tumors, the concentration of CXCL12 in the anti-CXCR4 group was significantly lower than that in the control group ($p < 0.0001$), while NSCLC had no intergroup CXCL12 difference ($p > 0.05$) (figure 7M). This supports CXCR4 inhibition disrupting CXCL12–CXCR4 interactions and reducing the chemotactic gradient for immunosuppressive cells, aligning with Xenium spatial transcriptomics and mIF data showing CXCR4⁺ neutrophil co-localization in SCLC.

Collectively, animal studies validated our in vitro findings that a hyperactive CXCL12–CXCR4 axis drives SCLC progression by recruiting immunosuppressive neutrophils. CXCR4 inhibition reduced tumor burden, correlating with decreased infiltration of CXCR4⁺ neutrophils and CXCL12 levels. This intervention reversed the spatial exclusion of CD8⁺ T cells by CXCR4⁺ neutrophils, thereby overcoming the immune-desert phenotype and identifying this axis as a promising immunotherapeutic target in SCLC (figure 7N).

Molecular subtyping of SCLC and heterogeneity in immune phenotypes

To contextualize our findings within the current SCLC subtype classification framework, we integrated our scRNA-seq data with public datasets^{31 32} to analyze four well-established SCLC subtypes: ASCL1-high (SCLC-A), NEUROD1-high (SCLC-N), POU2F3-high (SCLC-P), and YAP1-high (SCLC-Y), as well as the proposed inflammatory subtype (SCLC-I) described by Gay *et al.*³³

UMAP visualization first confirmed clear segregation of SCLC-A and SCLC-N subtypes (online supplemental Figure S11A), with a bubble plot identifying ASCL1/ISL1 as SCLC-A markers and NEUROD1 as the core marker

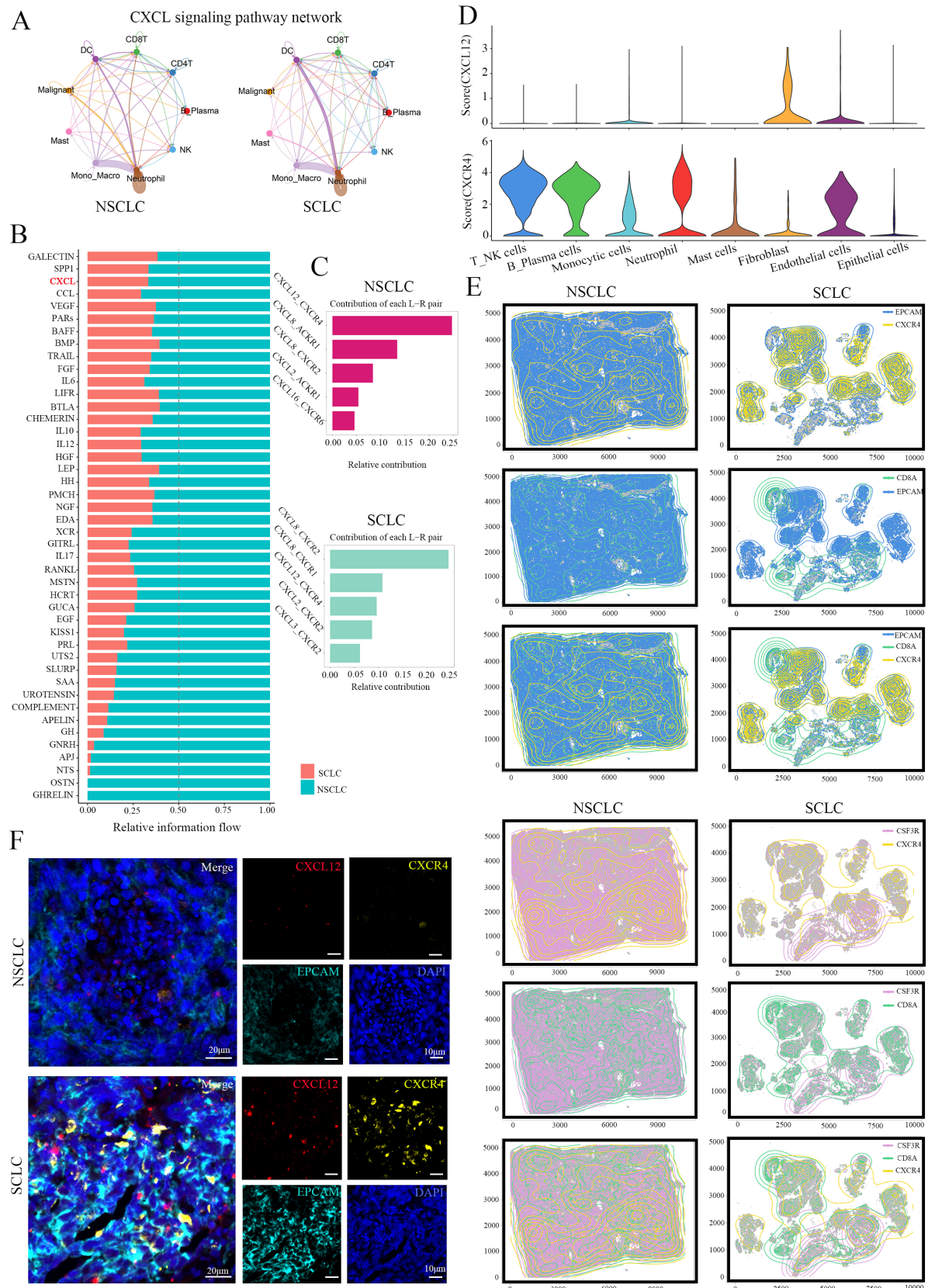


Figure 6 Competitive immunosuppressive mechanism mediated by the CXCL12–CXCR4 axis in SCLC. (A) Weighted graph of intercellular communication among different cell populations generated using CellChat. (B) RankNet ranking graph. (C) Contribution graph of ligand-receptor pairs. (D) Violin plot showing the expression scores of CXCL12 and CXCR4 in cell subpopulations. (E) Contour plot showing the distribution density of CXCL12⁺ and CXCR4⁺ cell subpopulations. (F) mIF staining of CXCL12⁺ and CXCR4⁺ epithelial cells. CXCL 12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C motif chemokine receptor 4; mIF, multiplex immunofluorescence; NK, natural killer; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

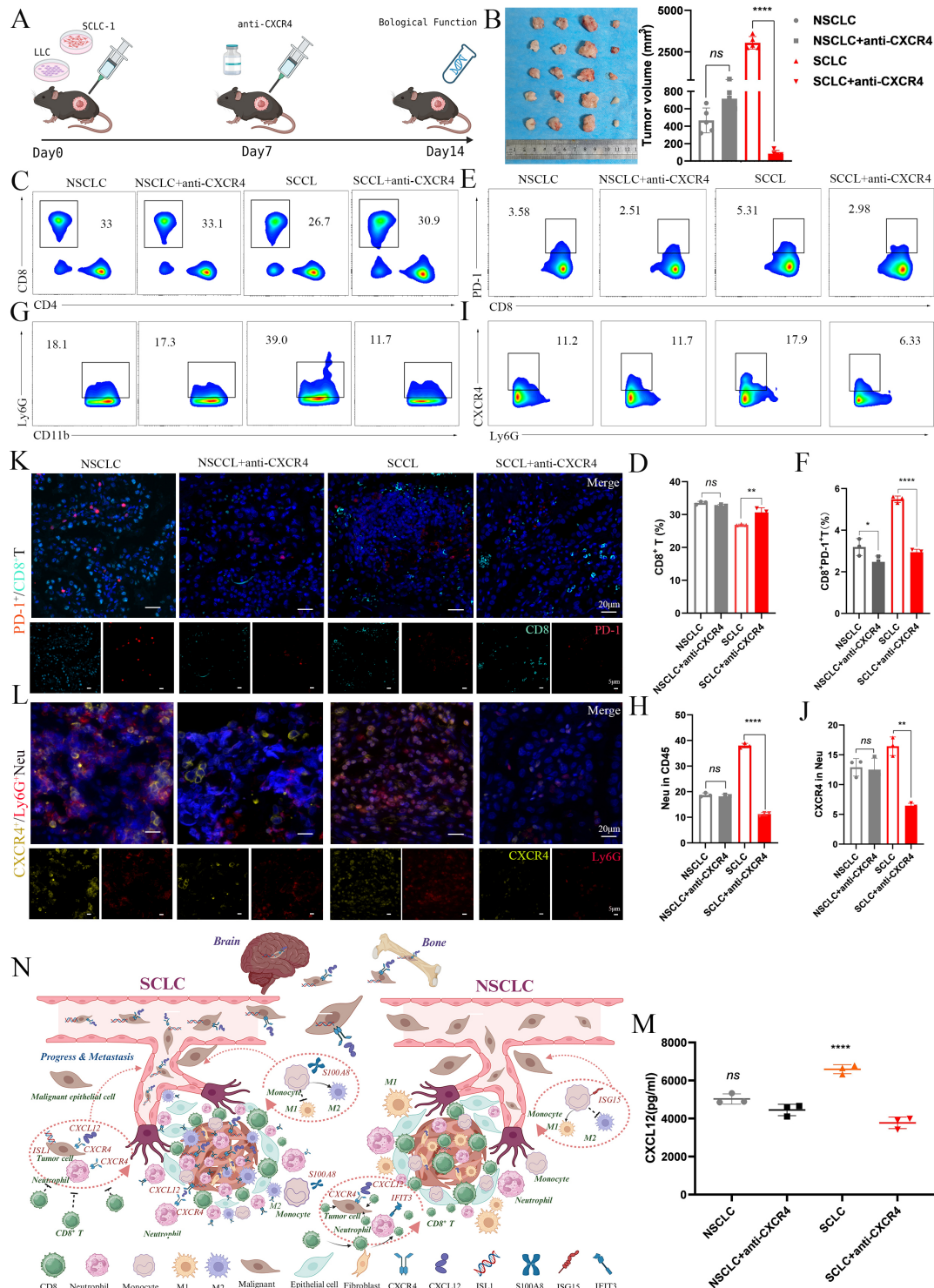


Figure 7 CXCR4 inhibition suppresses SCLC growth and reshapes the tumor immune microenvironment in mice. (A) Schematic illustration of the in vivo experimental procedure. (B) Growth curves of subcutaneous SCLC and NSCLC tumors in control and anti-CXCR4-treated mice. (C, D) Representative flow cytometry analysis and quantification of the proportion of CD8⁺ T cells and PD-1⁺ CD8⁺ T cells in SCLC and NSCLC groups. ****p<0.001. (E, F) Representative flow cytometry analysis and quantification of the proportion of Ly6G⁺ neutrophils and Ly6G⁺ CXCR4⁺ neutrophils in SCLC and NSCLC groups. *p<0.05, ****p<0.0001. (G) Representative MIF staining images of tumor sections for CD8⁺ T cells and PD-1⁺ CD8⁺ T cells from each group (green: CD8, red: PD-1) (Scale bar: 20 μm, 5 μm). (H) Representative pIF staining images of tumor sections for Ly6G⁺ neutrophils and Ly6G⁺ CXCR4⁺ neutrophils from each group (yellow: CXCR4, red: Ly6G) (scale bar: 20 μm, 5 μm). (I) Concentration of CXCL12 in tumor tissue supernatants from control and anti-CXCR4-treated mice. ****p<0.0001. (J) Schematic diagram of the mechanism for the formation of the immune desert phenotype in SCLC. CXCL 12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C motif chemokine receptor 4; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; SCLC, small cell lung cancer; MIF, Multiplex Immunofluorescence.

for SCLC-N (online supplemental Figure S11B).³³ UMAP plots further validated the specific expression of these signature genes across the two subtypes (online supplemental Figure S11C). For SCLC-I, defined by IRF9, IFI44, IFIT1, ISG15, MX1, and CXCL10³³, expression patterns showed poor subtype discrimination—these genes were barely detectable in SCLC samples (online supplemental Figure S11D). A bubble plot of SCLC subtype-associated genes further confirmed the enrichment of ASCL1/ISL1 in SCLC-A and NEUROD1 in SCLC-N, while SCLC-I markers failed to form a distinct cluster (online supplemental Figure S11E).

Expanding to three-subtype classification (A, N, L), UMAP plots showed persistent separation of SCLC-A and SCLC-N, with SCLC-L present at low abundance (online supplemental Figure S11F).³¹ Marker genes for the three subtypes were clearly delineated in a bubble plot (online supplemental Figure S11G), and their specific expression was validated by UMAP visualization (online supplemental Figure S11H). Again, SCLC-I markers showed inconsistent expression across the three subtypes (online supplemental Figure S11I), and subtype-associated genes confirmed the dominance of SCLC-A and SCLC-N signatures (online supplemental Figure S11J).

For four-subtype classification (A, N, P, Y), UMAP visualization revealed SCLC-A and SCLC-N as the dominant subtypes, SCLC-P at extremely low frequency, and SCLC-Y rarely detected in SCLC samples (online supplemental Figure S11K).³² A bubble plot detailed the marker genes for each subtype: ASCL1/ISL1 for SCLC-A, NEUROD1 for SCLC-N, POU2F3 for SCLC-P, and YAP1 for SCLC-Y (online supplemental Figure S11L), with UMAP plots confirming their specific expression (online supplemental Figure S11M). SCLC-I markers remained non-discriminatory (online supplemental Figure S11N), and subtype-associated genes reinforced the prominence of SCLC-A and SCLC-N (online supplemental Figure S11O).

Replicate UMAP visualization of the four-subtype framework (A, N, P, Y) consistently showed SCLC-A and SCLC-N dominance (online supplemental Figure S11P and Q), with marker gene expression validated by UMAP plots (online supplemental Figure S11R and S) in our study. A bubble plot of SCLC-I-associated genes confirmed their unstable expression across subtypes (online supplemental Figure S11T).

Cross-comparison between NSCLC and SCLC subtypes (A, N, P, Y) via UMAP visualization showed SCLC-Y was almost exclusively restricted to NSCLC (online supplemental Figure S12A), with a bubble plot confirming subtype-specific markers across both cancer types (online supplemental Figure S12B).³² Proportional composition analysis revealed SCLC-A and SCLC-N accounted for the majority of SCLC cells, while SCLC-P/Y were rare (online supplemental Figure S12C). Violin plots showed SCLC-I markers (IFI44, IFIT1, MX1, CXCL10) were highly expressed in NSCLC but barely detectable in SCLC, and antigen presentation-related genes (B2M, HLA-A, HLA-B, PSMB8) showed ubiquitous expression

across SCLC subtypes (online supplemental Figure S12D).

CXCR4, a key mediator of immunosuppression, was preferentially expressed in SCLC-A and SCLC-N, as shown by UMAP visualization (online supplemental Figure S12E). UMAP analysis of monocyte populations revealed distinct clustering between NSCLC and SCLC (online supplemental Figure S12F), with a bubble plot identifying marker genes for monocyte subsets (online supplemental Figure S12G) and proportional composition analysis showing higher abundance of S100A8⁺ monocytes (M2-polarized) in SCLC (online supplemental Figure S12H). UMAP visualization confirmed the presence of CXCR4⁺/S100A12⁺ neutrophil clusters (immunosuppressive neutrophils/myeloid-derived suppressor cells (MDSCs); online supplemental Figure S12I), with a bubble plot detailing their marker genes (online supplemental Figure S12J) and proportional analysis showing higher abundance in SCLC versus NSCLC (online supplemental Figure S12K).

CXCR4 expression and myeloid cell dynamics in metastatic and recurrent SCLC

To address SCLC metastasis, we analyzed CXCR4 expression and myeloid cell infiltration in primary versus metastatic lesions.³¹ UMAP visualization of metastatic SCLC subtypes (A, N, L) showed SCLC-A as the dominant subtype (online supplemental Figure S13A), with a bubble plot confirming marker genes for each subtype (online supplemental Figure S13B). Proportional composition analysis revealed a marked increase in SCLC-A cells in metastatic sites compared with primary lesions (online supplemental Figure S13C). Violin plots showed key subtype genes (ASCL1, NEUROD1) were retained in metastases, and CXCR4 expression was significantly higher in metastatic SCLC-A than primary SCLC-A (online supplemental Figure S13D and E).

UMAP visualization of monocyte populations in SCLC showed distinct clustering of subsets (online supplemental Figure S13F), with a bubble plot identifying their marker genes (online supplemental Figure S13G). Proportional composition analysis revealed a shift in metastases: increased S100A8⁺ monocytes (M2-polarized, immunosuppressive) and decreased pro-inflammatory ISG⁺ monocytes (M1-polarized) compared with primary lesions (online supplemental Figure S13H). UMAP plots showed marker gene expression in SCLC neutrophils (online supplemental Figure S13I) and their distinct distribution (online supplemental Figure S13J). UMAP analysis of neutrophil populations revealed increased infiltration in metastatic versus primary lesions (online supplemental Figure S13K), with proportional composition analysis showing reduced CD8⁺ T-cell infiltration in metastatic niches (online supplemental Figure S13L). UMAP visualization confirmed the presence of CXCR4[−] and CXCR4⁺ neutrophil populations (online supplemental Figure S13M), and proportional analysis showed

almost all metastatic neutrophils were CXCR4⁺ (online supplemental Figure S13N).

For recurrent SCLC, UMAP visualization of subtypes (A, N) showed SCLC-A remained the dominant subtype (online supplemental Figure S14A), with a bubble plot confirming marker genes (ASCL1 for SCLC-A, NEUROD1 for SCLC-N; online supplemental Figure S14B) and UMAP plots validating their specific expression (online supplemental Figure S14C).³² UMAP visualization of CXCR4 expression showed enriched signal in both recurrent SCLC-A and SCLC-N (online supplemental Figure S14D). UMAP comparison of cellular populations between primary and recurrent lesions revealed a significant increase in CXCR4⁺ cells in recurrence (online supplemental Figure S14E). Proportional expression analysis confirmed recurrent SCLC-A had a higher proportion of CXCR4⁺ tumor cells than primary SCLC-A, with SCLC-N showing a modest increase (online supplemental Figure S14F and G).

Together, these data demonstrate that SCLC-A and SCLC-N are the dominant subtypes with preferential CXCR4 expression; SCLC-I lacks robust discriminative power; and CXCR4 upregulation in SCLC-A (the dominant metastatic/recurrent subtype) and increased CXCR4⁺ neutrophil infiltration are key features of progressive SCLC, validating the clinical relevance of CXCL12–CXCR4-mediated immunosuppression.

DISCUSSION

SCLC is a highly aggressive neuroendocrine tumor with a tumor TME distinct from NSCLC, characterized by low immune infiltration and hijacked cell functions that foster a tumor-promoting milieu. High-lactate TME in SCLC drives T-cell exhaustion via monocarboxylate transporter 11, inducing a “cold-tumor” phenotype. Here, we integrated single-cell and spatial transcriptomic analyses to dissect SCLC/NSCLC TIME, identifying key immune landscape features underlying SCLC’s poor immunotherapy response, and highlighting mechanisms of its immune-desert phenotype and potential therapeutic targets, with a focus on translating these findings into clinical applications.

Neutrophil-mediated immunosuppression

Consistent with prior work, our scRNA-seq confirmed the immunosuppressive TME in SCLCs, characterized by reduced CD8⁺ T-cell infiltration, impaired antigen presentation. Notably, we observed an enrichment of two key immunosuppressive cell populations: CXCR4⁺ neutrophils (polymorphonuclear MDSCs) and S100A8⁺ monocytes (monocytic MDSCs). These cells are recruited via the CXCL12–CXCR4 axis, where they inhibit T cells and promote angiogenesis,³⁴ contribute to pre-metastatic niches, and drive chemotherapy resistance.³⁵ Their immunosuppressive activity is mediated through the activation of the S100A8/A9-TLR4/RAGE/CD147-NF-κB/STAT3 signaling pathway, leading to immune evasion.³⁶

In contrast, NSCLC had higher T/B cells and M1 macrophages (immune-infiltrated phenotype). Xenium spatial transcriptomics validated spatial segregation: SCLC tumor/immunosuppressive cells occupied niches separate from CD8⁺ T cells, while NSCLC tumor/immune cells intermingled—this segregation restricts immune surveillance and drives immunotherapy resistance.

Notably, SCLC harbored more than twofold neutrophils than NSCLC, with downregulated MHC class I (HLA-A/B/C) and reduced expression of phagocytosis/antigen presentation genes, reflecting a functional shift toward immunosuppression. Prior studies have established that IL-1β-induced IL-17 from γδ T cells drives neutrophil expansion and polarization in a granulocyte colony-stimulating factor-dependent manner to suppress CD8⁺ T cells,³⁷ and that tumor-infiltrating neutrophils induce IL-17A⁺ Th cells via B7-H2 to enhance cancer cell proliferation.³⁸ Our GO and pathway analyses extended these findings by linking SCLC’s neutrophil phenotype to upregulated IL-17/TNF-driven chemokine signaling—a pathway not only facilitates neutrophil recruitment but also impairs antitumor functions. Importantly, we demonstrated that SCLC neutrophils actively suppress T-cell activation, reinforcing the immune-desert phenotype that characterizes this disease.

Monocyte polarization and immune evasion

Monocyte polarization to immunosuppression is key for immune evasion.³⁹ In SCLC, S100A8⁺ monocytes reinforce immune evasion by S100A8/A9-RAGE/TLR4-NF-κB/STAT3 pathway, which drives M2 polarization and induces immunosuppressive mediators. Concurrently, they exhibit impaired antigen presentation and elevated pro-angiogenic factors (VEGF/HIF-1), collectively promoting angiogenesis and suppressing antitumor immunity.⁴⁰ In contrast, NSCLC-enriched ISG⁺ monocytes (C3) are pro-inflammatory: type I IFN-driven, linked to M1 polarization, tumor killing, and T-cell activation⁴¹, with IFIT3 amplifying type I IFN/NF-κB signaling to drive M1 polarization.⁴² Our findings reveal that in SCLC, an M1-to-M2 monocyte shift coupled with CXCL8/CXCR2-mediated neutrophil recruitment initiates a feedforward suppression loop. This loop is directly associated with a decline in tumor immunogenicity (reduced MHC class I) and profound CD8⁺ T cell impairment. The latter manifests as defective activation, an altered Teff/Tem balance, and a distinct exhaustion phenotype characterized by upregulated HAVCR2/TIGIT alongside downregulated CD3/CD8.

CXCL12–CXCR4 axis and ISL1/DLX5 regulation in SCLC

CXCR4⁺ epithelial cells secrete CXCL12 to recruit regulatory T cells (Tregs) and MDSCs and promote T-cell efflux and exhaustion. Tumor-associated lymphatic endothelial cell-secreted CXCL12 binds T-cell CXCR4, sequestering effector T cells to the tumor periphery.⁴³ The CXCL12–CXCR4 axis plays a central and multifaceted role in shaping the immunosuppressive TME of SCLC.

CXCL12 (stromal/tumor-derived) mediates physiological lymphocyte homing, but tumor over-secretion creates gradients that sequester CXCR4⁺ T cells in CXCL12-rich regions (eg, tumor margin), preventing parenchymal infiltration, suppressing T-cell function and recruiting Tregs/MDSCs.⁴⁴ This phenomenon is termed spatial sequestration.

Aggressive SCLC is driven by transcription factors ISL1 and DLX5, which define high-CXCR4 subsets. ISL1, a master regulator of neuroendocrine-high SCLC metastasis, directly activates CXCR4 transcription.⁴⁵ DLX5, core to ASCL1-high SCLC, is often co-expressed with ISL1 and CXCR4.⁴⁶ Through SCENIC analysis, we confirmed that ISL1 directly regulates CXCR4, mechanistically by binding the Set1/MI1 complex (eg, Wdr5) to activate CXCL12/CXCR4 transcription,⁴⁷ a mechanism that establishes a direct causal link between ISL1-driven oncogenic signaling and enhanced tumor aggressiveness. This connection is mediated by CXCL12/CXCR4-dependent recruitment of immunosuppressive cells, promotion of angiogenesis, and induction of T-cell exclusion, collectively fueling tumor progression and treatment resistance. Notably, chemokine receptor redundancy may contribute to the complexity of this pathway: CXCR7, another receptor for CXCL12, has been reported to form heterodimers with CXCR4 or act independently to mediate CXCL12-induced protumorigenic effects, such as cell survival and migration in various cancers.^{48,49} Although not the focus of the current study, the potential compensatory role of CXCR7 or other chemokine receptors (eg, CXCR2) cannot be excluded, highlighting the need for comprehensive evaluation of the CXCL12 chemokine network in future investigations to optimize therapeutic strategies. This makes the ISL1–DLX5–CXCR4 axis a promising therapeutic target.

Our analysis identified three distinct malignant epithelial subsets: SCLC-enriched C1/C2 clusters exhibited high CXCR4, ISL1, and DLX5 expression with reduced HLA and enriched hypoxia/proliferation pathways, whereas the NSCLC-enriched C3 cluster showed high HLA-A/B/ISG15 expression and active antigen presentation. Xenium spatial transcriptomics revealed that SCLC-derived EPCAM⁺ CXCR4⁺ epithelial cells are spatially segregated from CD8⁺ T cells, while CXCR4⁺ neutrophils colocalized with tumor cells. This spatial architecture suggests a dual role for CXCR4 signaling: (1) tumor-secreted CXCL12 recruits CXCR4⁺ neutrophils to suppress T-cell infiltration through competitive chemokine binding or direct inhibition, and (2) downregulated CXCL12–CXCR4 signaling within T cells themselves reduces their chemotaxis to tumors.

Our cell–cell communication analysis revealed a dysregulated chemokine landscape in SCLC: signaling through the CXCL12–CXCR4 axis (crucial for T-cell recruitment) is downregulated, while the CXCL8–CXCR2 (driving neutrophil recruitment) is upregulated. This imbalance mechanistically explains the observed reduction in T cells and increases in neutrophils. Spatial transcriptomic

data confirmed that CXCR4⁺ cells (both tumor cells and neutrophils) in SCLC occupy regions devoid of CD8⁺ T cells, a pattern distinct from NSCLC where more overlap is seen. This reveals that in SCLC, the CXCL12–CXCR4 not only fails to recruit T cells but actively excludes them through spatial competition—a mechanism that has not been previously described in detail.

SCLC subtype heterogeneity and immunological characteristics

Recent advances in SCLC molecular subtyping have defined four major subtypes based on lineage-specific transcription factors: ASCL1-high (SCLC-A), NEUROD1-high (SCLC-N), POU2F3-high (SCLC-P), and YAP1-high (SCLC-Y), alongside a proposed “inflamed” subtype (SCLC-I) characterized by immune infiltration.³³ To contextualize our findings within this framework, we integrated our single-cell data with three public datasets from public human datasets. Consistent across all datasets, SCLC-A was the dominant subtype with the highest proportional abundance, followed by SCLC-N; SCLC-P was rare, and SCLC-Y was predominantly associated with NSCLC. Notably, robust CXCR4 expression was detected in SCLC-A and SCLC-N, consistent with our earlier observation of CXCR4’s role in immunosuppression.

In contrast, enrichment for SCLC-I using its signature genes (IRF9, IFI44, IFIT1, ISG15, MX1, CXCL10) showed poor discriminatory ability: these genes were barely expressed in SCLC samples, with IFI44, IFIT1, MX1, and CXCL10 restricted to NSCLC. MHC class I-related genes (B2M, HLA-A, HLA-B, PSMB8) were ubiquitously expressed across SCLC subtypes and failed to distinguish SCLC-I, while IRF9 and ISG15 exhibited unstable expression in SCLC. Collectively, these data suggest the proposed SCLC-I subtype shares greater transcriptional similarity to NSCLC than to SCLC, with limited distinctiveness in SCLC cohorts.

Recurrence-focused analysis further revealed that SCLC-A is the primary recurrent subtype, with recurrent SCLC-A cells showing significantly higher CXCR4 positivity compared with primary cells. This subtype-specific CXCR4 upregulation highlights a potential mechanism driving the aggressive recurrence of SCLC-A and underscores the necessity of subtype-stratified therapeutic strategies targeting the CXCL12–CXCR4 axis.

The spatial competition mechanism between CXCR4⁺ tumor/immunosuppressive cells and CD8⁺ T cells in SCLC

A most innovative finding of this study is the revelation of the spatial competition mechanism between CXCR4⁺ tumor/immunosuppressive cells and CD8⁺ T cells, providing a novel academic explanation for the formation of the immune-desert phenotype in SCLC. Unlike the intermingling of tumor cells and immune cells in NSCLC,⁵⁰ Xenium spatial transcriptomic data clearly demonstrate that CXCR4⁺ tumor cells and CXCR4⁺ neutrophils (immunosuppressive myeloid cells) in SCLC co-occupy specific TME niches, and these regions are

completely devoid of CD8⁺ T-cell infiltration. The core mechanism underlying this spatial distribution pattern lies in the aberrant regulation of the CXCL12–CXCR4 axis: excessive secretion of CXCL12 by tumor cells and stromal cells forms a chemical gradient. On one hand, it competitively binds to CXCR4 on the surface of CD8⁺ T cells, sequestering effector T cells in CXCL12-rich regions (eg, tumor margins) and preventing their infiltration into the tumor parenchyma; on the other hand, this gradient efficiently recruits CXCR4⁺ immunosuppressive cells (neutrophils, MDSCs, etc) into the core tumor region, further forming physical and functional immune barriers. This spatial competition mechanism not only explains the key reason for insufficient CD8⁺ T-cell infiltration in SCLC but also breaks through the traditional understanding that immune exclusion relies solely on molecular-level signal inhibition.⁵¹ It clarifies the central role of spatial occupancy competition in tumor immune escape, providing a novel perspective for understanding the low responsiveness of SCLC to ICIs and holding significant academic innovative value.

CXCR4-mediated immunosuppression in recurrent and metastatic SCLC

A key clinical hallmark of SCLC is early recurrence and distant metastasis, despite initial sensitivity to chemotherapy, underscoring the need to validate our TME findings in these aggressive disease settings. Leveraging public human datasets,³¹ we analyzed CXCR4 expression and myeloid cell characteristics in primary vs recurrent/metastatic SCLC. For metastatic lesions, three key observations were made: (1) the proportion of SCLC-A (the CXCR4-high subtype) was significantly increased compared with primary tumors; (2) metastatic lesions exhibited marked neutrophil accumulation, with nearly all metastatic neutrophils expressing high levels of CXCR4; (3) the spatial segregation of CXCR4⁺ cells (tumor and neutrophils) from CD8⁺ T cells—observed in primary SCLC—was further exacerbated in metastases.

In recurrent SCLC, CXCR4 positivity was consistently higher in recurrent tumor cells (especially SCLC-A) than in primary counterparts, with recurrent lesions showing enhanced recruitment of CXCR4⁺ neutrophils, consistent with the patterns observed in metastatic disease.³² These data extend our primary tumor findings, demonstrating that CXCR4-driven immunosuppression (via neutrophil recruitment and T-cell exclusion) is amplified in recurrent and metastatic SCLC, and is likely a key contributor to treatment resistance in these settings. Importantly, this aligns with preclinical studies showing that CXCR4 inhibition reduces SCLC growth by targeting neutrophil infiltration, suggesting that CXCR4 inhibitors may be particularly effective in recurrent/metastatic SCLC, where CXCR4-mediated immunosuppression is most pronounced.

Therapeutic implications and preclinical validation

In SCLC, the inflammation-induced and tumor/tumor-associated macrophage (TAM)-derived chemokine CXCL8 recruits neutrophils.⁵² These neutrophils polarize into a protumor N2 phenotype under the influence of transforming growth factor-beta (TGF-β), IL-6, IL-10, and IL-1β,⁵³ forming physical barriers and promoting angiogenesis and epithelial-mesenchymal transition.⁵⁴

CXCR4 inhibitors, by blocking CXCL12–CXCR4 axis, can reshape the TME: they alleviate T-cell exclusion, reduce suppressive cells, and enhance dendritic cell and vascular function. This makes them ideal candidates for combination with ICIs to overcome the resistance stemming from insufficient T-cell infiltration.⁵⁵ Beyond therapeutic strategy, our findings also provide critical guidance for biomarker development: the abundance of CXCR4⁺ cells (tumor cells and immunosuppressive myeloid cells) or their spatial segregation from CD8⁺ T cells could serve as predictive biomarkers for ICI efficacy in SCLC. Patients with high CXCR4⁺ cell infiltration or prominent spatial exclusion of CD8⁺ T cells may be more likely to benefit from CXCR4 inhibitor-ICI combinations, enabling precise patient stratification. Additionally, dynamic monitoring of CXCR4 expression or spatial TME features during treatment could help assess response and adjust therapeutic regimens in real time.

Early-phase clinical trials support this approach. For instance, combinations like BL-8040 with pembrolizumab in pancreatic cancer⁵⁶ and mavorixafor with nivolumab in clear cell renal cell carcinoma⁵⁷ have shown improved disease control, response rates, and survival in ICI-resistant or low-immune patients. However, several challenges must be addressed for successful clinical translation. First, potential toxicities and off-target effects of CXCR4 inhibitors need careful consideration: CXCR4 is physiologically expressed in hematopoietic stem cells, lymphocytes, and endothelial cells, so systemic inhibition may cause myelosuppression, lymphopenia, or vascular complications.⁴⁴ Second, drug delivery remains a hurdle—achieving sufficient concentrations of CXCR4 inhibitors in the tumor core while minimizing systemic exposure is critical to balance efficacy and safety. Third, intrinsic or acquired resistance to CXCR4 blockade may emerge, possibly driven by redundant chemokine receptors (eg, CXCR7)⁵⁸ or alternative immunosuppressive pathways (eg, TGF-β signaling).⁵⁹ Fourth, optimal patient stratification biomarkers are lacking; current trials often enroll unselected patients, leading to variable response rates. Addressing these issues requires collaborative efforts in preclinical drug optimization, clinical trial design, and companion diagnostic development.

Based on prior scRNA-seq and Xenium data, we established mouse LC models to validate the therapeutic role of CXCR4. In these models, CXCR4 inhibitors selectively reduced tumor growth in SCLC but not in NSCLC. This antitumor effect was accompanied by a decrease in neutrophil infiltration, lower CXCR4 expression, and reduced CXCL12 secretion specifically in SCLC tumors.

These results confirm that the CXCL12–CXCR4 axis is a key driver of immune-desert phenotype in SCLC, thereby establishing it as a rational target for combination therapy.

To visually summarize the core immunosuppressive mechanisms of SCLC's immune-desert phenotype, we constructed a schematic diagram (online supplemental Figure S15) integrating key molecular and cellular interactions. As illustrated, transcription factors ISL1 and DLX5 drive malignant epithelial cells to secrete CXCL12, which activates the CXCL12–CXCR4 axis to recruit two critical immunosuppressive populations: CXCR4⁺ neutrophils (via NETs) and S100A8⁺ M2-like monocytes. CXCR4⁺ neutrophils downregulate MHC-I to impair antigen presentation and CD8⁺ T-cell activation, while S100A8⁺ monocytes weaken antigen presentation and enhance angiogenesis via VEGF/HIF-1. Concurrently, the CXCL12 gradient mediates spatial competition—sequestering CD8⁺ T cells at the tumor periphery and preventing their infiltration into the core, where CXCR4⁺ tumor cells and immunosuppressive cells form physical/functional barriers. This multilayered network collectively drives SCLC's immune-desert phenotype. Notably, CXCR4 inhibition disrupts this axis, reducing immunosuppressive cell infiltration, restoring CD8⁺ T-cell accumulation, and curbing tumor growth—highlighting the therapeutic potential of targeting this pathway to remodel the TME and enhance immunotherapy efficacy.

Conclusion and future directions

This study elucidates the mechanistic basis for the low response rate to ICI in SCLC. Unlike in NSCLC, where high PD-L1 or tumor mutational burden predicts ICI benefit, the immune-desert phenotype in SCLC primarily stems from defective T-cell infiltration and the accumulation of immunosuppressive myeloid cells, rather than from dominant PD-1/PD-L1 activation. Therefore, strategies aimed at normalizing the TME may act synergistically with ICIs to improve therapeutic efficacy. For example, CXCR4 blockade can be employed to limit the recruitment of immunosuppressive neutrophils or monocytes could be reprogrammed toward an immunostimulatory M1 polarization. Furthermore, we identify the transcription factor ISL1, a direct regulator of CXCR4 in SCLC, as a promising upstream target. Inhibiting ISL1 could simultaneously reduce CXCR4 expression in both tumor cells and immunosuppressive cells, thereby addressing multiple suppression mechanisms. However, our study is limited by the relatively small sample size and absence of longitudinal data to monitor TME evolution during treatment. Future work requires validation in larger patient cohorts, analysis of matched pretreatment and post-treatment samples, and functional assays to strengthen the established causal links.

Despite these insights, our study has several limitations that warrant consideration. First, the relatively small sample size and lack of longitudinal data limit the generalizability of our findings and prevent us from monitoring TME dynamics during treatment. Second, we did not

perform multi-omics integration analysis—for example, proteomic validation of key molecules (eg, CXCR4, ISL1, S100A8/A9) was not conducted to confirm translational expression at the protein level, and epigenetic profiling could have provided additional mechanistic insights into CXCL12/CXCR4 regulation. Third, functional experiments targeting specific immune subsets (eg, neutrophil depletion or monocyte polarization assays) were not performed to directly validate their immunosuppressive roles in SCLC. Fourth, our *in vivo* validation was limited to a single mouse LC model; testing in models with different SCLC genetic backgrounds (eg, TP53/RB1 double knockout, MYC-amplified) is necessary to confirm the universal relevance of the CXCL12–CXCR4 axis. Fifth, the potential crosstalk between CXCR4 signaling and other immune regulatory pathways (eg, type I IFN, complement system) was not explored, which may limit our understanding of the full complexity of the SCLC TME.

Future work should address these limitations and test novel hypotheses derived from our study. For instance, we hypothesize that targeting ISL1 or CXCR4 may synergize with type I IFN pathway activation (eg, via STING agonists) or myeloid cell reprogramming (eg, M2-to-M1 monocyte conversion) to further enhance antitumor immunity. Additionally, combining CXCR4 inhibitors with other TME-modulating agents (eg, anti-TGF- β antibodies, VEGF inhibitors) could disrupt multiple immunosuppressive pathways and improve treatment response. Longitudinal multi-omics studies in larger patient cohorts are needed to validate CXCR4-related biomarkers and monitor TME evolution during combination therapy. Furthermore, the development of tumor-specific CXCR4-targeted delivery systems (eg, antibody-drug conjugates, nanoparticle-based formulations) may reduce off-target toxicities and improve therapeutic index. Finally, functional studies using patient-derived organoids or xenografts will help clarify the cell-intrinsic and cell-extrinsic mechanisms underlying CXCR4-mediated immune exclusion and guide the design of more effective combination strategies.

In conclusion, our integrated single-cell and spatial transcriptomic analyses reveal that the immune-desert phenotype in SCLC is a multifaceted condition. It is driven by tumor-intrinsic oncogenic signaling through the CXCR4/ISL1 axis, the pathological accumulation of immunosuppressive neutrophils and M2-polarized monocytes, and the physical spatial segregation of immune cells. Therapeutic strategies aimed at disrupting the CXCL12–CXCR4 axis or targeting its upstream regulators hold significant potential to dismantle these barriers, thereby paving the way for more effective immunotherapies in SCLC.

METHODS

Fresh tumor tissues were obtained from untreated patients with LC who underwent transbronchoscopic

biopsy. All samples were pathologically confirmed. Tumor tissues from five patients with SCLC and four patients with NSCLC were dissociated into single-cell suspensions for scRNA-seq using the 10x Genomics platform. Cell clustering, annotation, and differential gene expression analysis were performed to identify TIME components and SCLC-specific features. Formalin-fixed, paraffin-embedded tissues from four patients with SCLC and four patients with NSCLC were analyzed using the Xenium platform to map spatial distribution of cell types and gene expression domains identified in scRNA-seq. Protein expression of key targets was validated in patient and mouse xenograft tissues. C57BL/6 mice bearing subcutaneous tumors were treated with a CXCR4 antagonist to assess tumor growth and changes in lymphocyte subsets. The clinical characteristics of all cases, including age, sex, histological type, histological category, and pathological stage, are summarized in online supplemental Table S3. The technological details of scRNA-seq and Xenium in situ spatial transcriptomics analysis, mIF assays and in vivo animal experimental research will be found in online supplemental materials.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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