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Spatial and single-cell transcriptomic profiling reveals interplay between SRGN-associated epithelial programs and C1QC+ macrophages in early LUAD progression

Shengqiang Mao^{1†}, Chunwei Pang^{2†}, Hong Hang¹, Yalun Li^{1*} and Li Zhang^{1*} 

Abstract

Background Deciphering the molecular and cellular mechanisms underlying the progression of early-stage lung adenocarcinoma (LUAD) is critical for advancing early detection and therapeutic strategies. This study aims to map the evolutionary trajectory from precursor lesions to invasive adenocarcinoma.

Methods We integrated single-cell RNA sequencing (scRNA-seq) and spatial transcriptomic analyses to evaluate LUAD progression across four histologic stages: atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and invasive adenocarcinoma (IAC). Findings were further validated using a stage-specific A/J mouse model to confirm functional cellular interactions.

Results We identified a tumor-intrinsic program characterized by elevated *SRGN* expression that is associated with Epithelial–Mesenchymal Transition (EMT) and cell proliferation, intensifying as the disease progresses. In parallel, a stage-dependent accumulation of C1QC+ macrophages was observed. Spatial colocalization analysis revealed that SRGN+ malignant cells and C1QC+ macrophages are closely positioned within the tumor microenvironment. We detected progressively enhanced interactions between SRGN+ malignant cells and both exhausted T cells and C1QC+ macrophages, mediated by specific receptor–ligand pairs, including *MIF–LIFR*, *ADAM17–ERBB4*, *OSM–EGFR*, and *OSM–TNFRSF14*. The A/J mouse models confirmed the co-emergence and functional cooperation of these populations.

Conclusions Our findings highlight a coordinated epithelial–macrophage module that drives LUAD progression. The identification of *SRGN* and its associated spatial interactions offers potential molecular targets for early therapeutic intervention and diagnostic modeling.

Keywords Lung adenocarcinoma, SRGN, LUAD progression

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Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide [1]. This is primarily because it is often diagnosed at advanced stages, when curative treatments are no longer effective [2–5]. Understanding the molecular and cellular events that drive early lung carcinogenesis is therefore essential for improving early detection, diagnosis, prevention, and intervention strategies [6–8]. However, such investigations are particularly challenging in LUAD, the most common and lethal subtype of lung cancer, owing to the limited availability of early-stage lesions.

LUAD is thought to arise through a stepwise histological continuum, transitioning from atypical adenomatous hyperplasia (AAH) to adenocarcinoma in situ (AIS), minimally invasive (MIA), and ultimately invasive adenocarcinoma (IAC) [9–11]. According to the 2021 WHO classification, AIS, a type of lesion that often presents as ground-glass opacity, is no longer categorized as LUAD, given its indolent behavior and favorable long-term prognosis compared with MIA and IAC [12–15]. While previous studies have focused primarily on the immune microenvironment at the invasive stage, the cellular and immunological changes that accompany LUAD initiation and early invasion remain poorly understood. Canonical oncogenic mutations, including those in *EGFR* and *TP53*, have been identified as early as the AIS stage and are believed to facilitate progression to IAC via evolutionary branching and activation of proinvasive transcriptional programs [16–18]. Moreover, disruption of immune surveillance has been observed in early lesions, highlighting the critical role of the immune microenvironment even during preinvasive stages.

The initiation and progression of tumors is a multistep process involving dynamic interactions between malignant cells and the surrounding immune landscape [19, 20]. These bidirectional communications shape a complex network that modulates both antitumor immunity and tumor-promoting inflammation. Although bulk RNA sequencing has provided important insights into the transcriptomic landscape of preinvasive LUAD, it lacks the ability to resolve cell type-specific signals because of the heterogeneous mixture of epithelial, stromal, and immune cells within tumors [21, 22]. As such, the composition, functional state, and spatial organization of immune and epithelial cells during LUAD initiation and early progression remain incompletely characterized [18, 23].

To address this knowledge gap, high-resolution technologies such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) are essential [24–26]. scRNA-seq enables unbiased profiling of heterogeneous cellular populations and dissection of lineage-specific

programs across developmental or pathological states [27, 28]. In parallel, ST captures gene expression along with spatial context, offering an integrated view of tissue architecture and cell–cell interactions during disease evolution [29]. Together, these approaches offer powerful tools to decipher the cellular ecosystems and intercellular communication networks that govern early LUAD progression [16, 30].

In this study, we constructed a comprehensive spatiotemporal atlas of early LUAD progression by integrating single-cell RNA sequencing and spatial transcriptomics across the full continuum from AAH to IAC. We identified a tumor-intrinsic epithelial program marked by SRGN+ malignant cells, a subpopulation characterized by high expression of *Serglycin*, which is associated with tumor invasiveness and pro-inflammatory signaling. We also revealed their spatial colocalization with immunosuppressive C1QC+ macrophages, a specific cluster of tumor-associated macrophages defined by complement component expression and terminal myeloid differentiation. Both of which expand progressively during LUAD evolution. Notably, these two cell populations engage in dynamic and reciprocal crosstalk, which is particularly pronounced at the AIS–MIA transition and promotes EMT and malignant progression. Together, our findings highlight a cooperative epithelial–macrophage axis that shapes early tumor development and represents a potential target for early diagnosis and therapeutic intervention in LUAD.

Materials and methods

Data acquisition and preprocessing

The single-cell RNA sequencing (scRNA-seq) datasets were obtained from a publicly available dataset (GEO: GSE189357) and from our previous study investigating early lung cancer progression (GSA: HRA001130). The spatial transcriptomics (ST) dataset was downloaded from the GEO database (GSE189487).

A/J mouse model

All A/J mice were procured from the Shanghai Model Organisms Center (Shanghai, China). Mice were randomized into groups and housed under a standard 12 h light/dark cycle with ad libitum access to food and water. At designated time points corresponding to various ages, mice were euthanized by decapitation. Following euthanasia, a thorough macroscopic examination of major organs was performed. Lung tissues were carefully dissected, with portions allocated for fixation and others processed into single-cell suspensions for downstream analyses. All procedures involving animals were reviewed and approved by the Ethics Committee for Animal Experiments at West China Hospital, Sichuan University.

Library preparation and sequencing

The tissue samples were washed with Hank's balanced salt solution (HBSS), minced into small fragments on ice, and enzymatically dissociated using a solution of collagenase I/IV in HBSS. The digestion was performed at 37 °C for 30 min with gentle manual agitation every 5 min. Following digestion, the cell suspension was centrifuged at 500 × g for 5 min. The cell pellet was resuspended in red blood cell lysis buffer, incubated briefly to remove erythrocytes, and then washed again with HBSS. Single-cell suspensions were processed via the Chromium Single Cell 3' Reagent Kit v3 (10x Genomics) according to the manufacturer's protocol. Barcoded gel beads and individual cells were encapsulated into emulsion droplets via the Chromium Controller, where cell lysis and reverse transcription occurred. The subsequent steps included cDNA amplification, fragmentation, and ligation of 5' adaptors and sample indices. The resulting libraries were sequenced on the Illumina NovaSeq 6000 platform at West China Hospital.

The scRNA-seq data clustering, dimension reduction and cell annotation

The “Seurat” package was used for single-cell analysis, and the data were integrated using the Harmony algorithm [31–33]. We first applied log normalization to the merged single-cell dataset and identified the top 2,000 highly variable genes via the FindVariableFeatures function with the “vst” method. All genes were scaled via ScaleData, and PCA was performed on the variable genes via RunPCA. The first 30 principal components were used for clustering with “FindNeighbors” and “FindClusters” (resolution = 0.5). UMAP was then applied to the 30 PCs for dimensionality reduction and visualization. We used FindAllMarkers to identify marker genes for each cluster, with log fold change > 0.25, minimum expression min.pct > 0.25, and adjusted p_value < 0.05.

Pseudotime and trajectory analysis

Pseudotime and trajectory analyses of epithelial cells were performed via the “Monocle3” package. This algorithm employs a machine learning-based approach to learn a parsimonious principal graph that maps high-dimensional gene expression data onto a low-dimensional space [34]. Individual cells were projected into this space and ordered along developmental trajectories with identifiable branch points, representing potential cell fate decisions [35]. DEGs across distinct epithelial cell states were identified via thresholds of a $|\log_2\text{-fold change}| > 0.5$ and a false discovery rate (FDR) < 0.05 and were considered key regulators of LUAD progression. To gain insights into the biological functions associated with these DEGs, Gene Ontology (GO) enrichment analysis

was conducted via the “clusterProfiler”, “org.Hs.eg.db”, “enrichplot”, and “ggplot2” packages [35]. Only biological process (BP) terms were included in the downstream interpretation.

Estimation of the activity of diverse signatures and pathways

The GSVA algorithm was implemented to evaluate the relative activation status for a signature or pathway [36]. GSVA scores for hallmark signatures, as well as signaling paths, were calculated via predefined gene sets extracted from the MSigDB.

Cellular communication analysis

The CellChat is a novel toolkit used to infer intercellular communication networks from scRNA-seq data quantitatively [37, 38]. Based on a human ligand–receptor interaction database and pattern recognition approaches, CellChat can predict major signaling inputs and outputs for cells and establish how these cells and signals coordinate their functions. The ligand–receptor pairs with a p value < 0.05 were filtered to evaluate the relationships between different cell types.

Cell-type identification by estimating relative subsets of RNA transcripts

The CIBERSORTx is a computational method that deconvolutes bulk gene expression profiles to estimate the relative proportions of distinct immune cell subsets within complex tissues. In this study, we applied CIBERSORTx to assess immune cell composition across different sample groups. To investigate the relationship between immune infiltration and disease risk, Spearman correlation analysis was conducted between the calculated risk scores and the estimated abundances of immune cell populations. Data visualization, including comparisons of immune cell proportions and correlation results, was performed via the “ggplot2” package.

Statistical analysis

All of the statistical analyses were conducted via R software (version 4.2.0). The Wilcoxon test was used to compare the differences between these two risk groups. $p < 0.05$ was regarded as statistically significant.

Cell lines and cell culture

The human lung fibroblast cell line MRC-5 and LUAD cell lines (HCC95, H1975, PC-9, and A549) were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). These cell lines were maintained under standard culture conditions in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; PAA Laboratories GmbH,

Pasching, Austria). All the cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂.

RNA interference

Short-interfering RNAs (siRNAs) targeting SRGN were purchased from Paiwei Biotechnology Co., Ltd. (Wuhan, China) and transfected into A549 cells via Lipofectamine 2000 (Invitrogen, Shanghai, China) following the manufacturer's instructions. Scrambled siRNA served as a negative control. Forty-eight hours after transfection, the efficiency of gene knockdown was assessed via Western blot analysis.

Cell invasion and migration assays

Invasion and migration assays were conducted using Transwell chambers (Corning, USA) coated with Matrigel for invasion or left uncoated for migration. The cells were resuspended in medium supplemented with 2% FBS and seeded into the upper chamber, while medium supplemented with 20% FBS was added to the lower chamber as a chemoattractant. After incubation at 37 °C for 24–48 h, nonmigrated/invaded cells on the upper surface were removed. The cells that passed through the membrane were fixed in methanol, stained with hematoxylin and eosin, and counted in three random fields (100× magnification). Each assay was independently performed in triplicate.

Multiplex immunohistochemistry (mIHC) of A/J mice

Mouse lung tissue sections were blocked with 10% normal goat serum and then incubated overnight at 4 °C with primary antibodies. After washing, the slides were incubated with an HRP-conjugated secondary antibody (goat anti-rabbit IgG H&L, 1:2000, ab205718, Abcam) at room temperature for 45 min. For multiplex IHC on 4 µm FFPE clinical sections, the Opal Polaris 7-Color Manual IHC Kit (Akoya, Cat# NEL861001KT) was used following dewaxing and rehydration. The primary antibodies used included anti-SRGN (A25426, ABclonal) and anti-C1QC (ab75756, Abcam).

mIHC statistical analysis

Multiplex immunohistochemistry images were acquired using the PANNORAMIC digital slide scanner. Quantitative analysis was performed with the High Plex FL module (v3.1.0) of Halo software (v3.0.311.314, Indica Labs). Regions of interest (ROIs) were manually selected, and the number of marker-positive cells within each ROI was quantified. Cell counts for each phenotype were further verified using Inform software. Cell density (cells/mm²) was calculated by normalizing the number of positive cells to the corresponding tissue area (in pixels) on each slide.

Results

scRNA-seq analysis of LUAD lesions reveals dynamic changes in the tumor microenvironment

We collected early-stage LUAD single-cell RNA sequencing (scRNA-seq) data from a public dataset. In total, 23 patient samples were included, comprising 3 with atypical adenomatous hyperplasia (AAH), 5 with adenocarcinoma in situ (AIS), 8 with minimally invasive adenocarcinoma (MIA), and 7 with invasive adenocarcinoma (IAC), along with 9 normal lung tissue samples (Fig. 1A). After integration using the Harmony package, cells from different datasets were well mixed, indicating successful batch correction (Fig. 1B). Following quality control, 220,057 cells were retained, including 58,212 from normal samples, 5,032 from AAH, 41,409 from AIS, 48,783 from MIA, and 66,621 from IAC (Fig. 1B). Nine major canonical cell types were identified, including epithelial cells, T cells, NK cells, B cells, plasma cells, myeloid cells, mast cells, fibroblasts, and endothelial cells (Fig. 1B). To explore tumor microenvironment (TME) remodeling during LUAD progression, we calculated the relative fractions of these cell types across stages (Fig. 1C). Notably, the proportions of B cells and plasma cells increased, while NK cells decreased from AAH to IAC, suggesting that immune composition changes dynamically during tumor evolution (Fig. 1D). Marker gene expression confirmed the identity of each cell type and enabled spatial localization (Fig. 1E). Together, these findings highlight the cellular heterogeneity and immune remodeling that accompany LUAD progression.

Cell type-specific transcriptomic reprogramming reveals key molecular transitions during early LUAD invasion

In addition to cell type composition heterogeneity, we further deciphered the functional differences in the specific pairs of cell types between different pathological subtypes. To further explore the cellular mechanisms underlying LUAD invasion, we compared the gene expression patterns of each cell type, including component cells from single-cell annotations, between the two groups. In addition, the same method was used to find functional differences in cell types between MIA and AIS patients (MIA vs. AIS) and between IAC and MIA patients (IAC vs. MIA) (Fig. 2A). During the invasive progression of LUAD, differential expression analysis was performed using a pseudo-bulk approach within each cell type. 512 upregulated and 332 downregulated DEGs were identified between the MIA and AIS groups, 199 upregulated and 347 downregulated DEGs were identified between the IAC and MIA groups, and 393 upregulated and 345 downregulated DEGs were identified between the IAC and AIS groups.

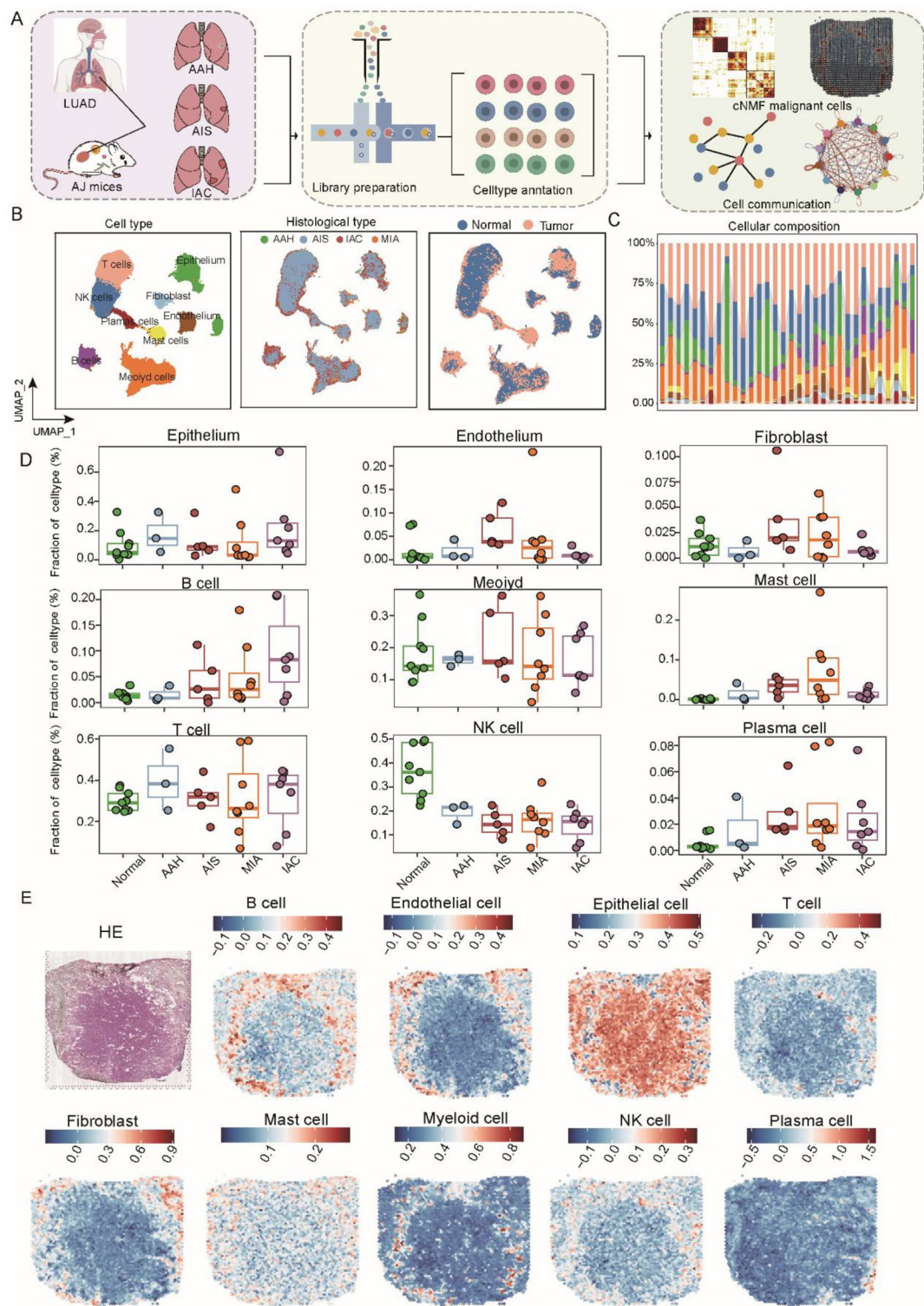


Fig. 1 Single-cell and ST profiling of lung biopsies revealed AAH to AIS, MIA, and IA. **(A)** Schematic diagram illustrating the workflow of lung adenocarcinoma biopsy processing for scRNA-seq and ST analyses. **(B)** UMAP representation of single cells profiled in the present work, colored by major cell type (left), pathological tissue subtype, and sample type (right) **(C)** Bar plots showing the proportion of cell types included in each individual for scRNA-seq. **(D)** Boxplot showing the cellular fractions of T, natural killer (NK), B, and myeloid cells and neutrophils in AAH, AIS, MIA, and IAC patients. **(E)** ST feature plots of P1 showing the expression of marker genes by annotated cell type

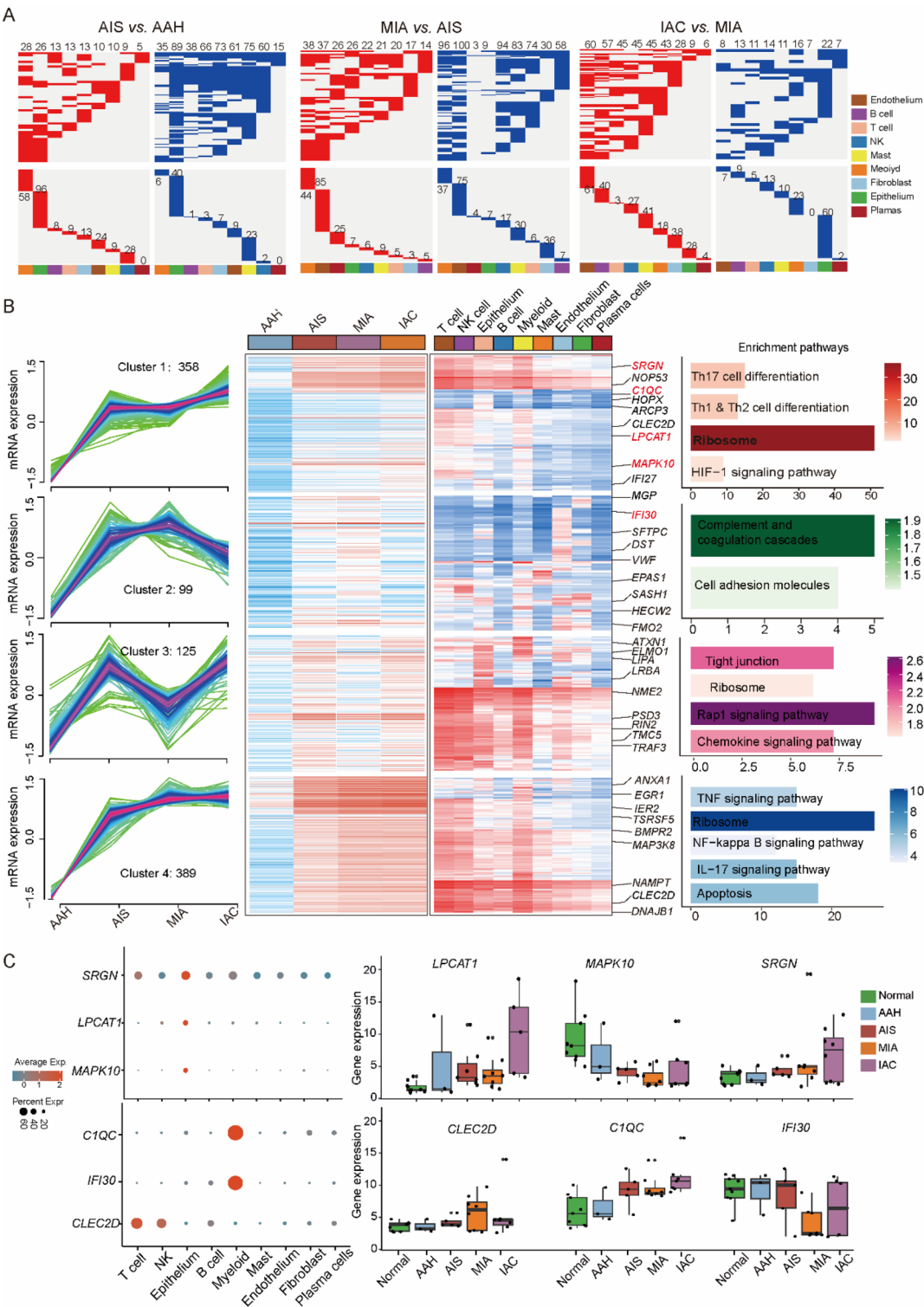


Fig. 2 Changes in the transcriptional profiles of different cell types among AAH, AIS, MIA, and IAC LUAD. **(A)** Heatmaps showing the distribution of upregulated (red) and downregulated (blue) genes. The upper part (dotted lines) represents the DEGs shared by at least two cell types, the lower panel represents the unique DEGs of each cell type, and the number below the dotted box represents the number of unique DEGs in each cell type. **(B)** Fuzzy clustering (Mufzz) analysis of the expression profiles of DEGs for each cell type from the different pathological subtype enrichment pathways on the basis of pseudobulk expression data and their role in GO function. MF, molecular function; BP, biological process; CC, cell component. **(C)** Dot plots depicting how the relative expression of particular genes varies among AAH, AIS, MIA, and IAC patients. The size displays the values for expression in the cell types, and colors are marked as up- or downregulation in the bulk cohort (red, increased; blue, decreased)

Next, the four transcriptomic profiling clusters were identified on the basis of the common DEG transcriptomic features (Fig. 2B). Cluster 1 (358 genes) was enriched for pathways related to Th17 cell differentiation, Th1 and Th2 cell differentiation, and the HIF-1 signaling pathway. Cluster 4 (389 genes) was characterized by high expression of genes involved in the TNF and NF- κ B signaling pathways, with progressive upregulation of apoptosis-related genes across disease stages. Notably, the expression of Cluster 4 and Cluster 1 genes gradually increased across the AAH to IAC subtypes. Cluster 2 (99 genes) exhibited high expression in the AIS and MIA subtypes, with enrichment in complement-related pathways and cell adhesion molecule pathways. Cluster 3 (125 genes) showed elevated expression primarily in the AIS subtype and was associated with pathways related to tight junctions, ribosome function, and chemokine signaling (Fig. 2B). Next, we screened for the most significantly differentially expressed genes in different cell types (ranked by foldchange).

To further determine the specific cell types enriched for these highly expressed genes, we examined their distribution across different cell types. The genes *SRGN*, *LPCAT1*, and *MAPK10* were highly expressed in epithelial cells and *C1QC*, *IFI30* were highly expressed in macrophages, and *CLEC2D* were highly expressed in T cells and NK cells (Fig. 2C). *SRGN* is upregulated in LUAD and promotes tumor progression through immune modulation, increased invasiveness, and epigenetic activation. As previously reported, *LPCAT1* was consistently upregulated and *MAPK10* downregulated from AAH to IAC, indicating enhanced membrane function and reduced apoptosis during LUAD progression [39, 40]. Meanwhile, *C1QC*+ macrophages exhibit an M2-like phenotype and promote tumor progression in lung cancer by enhancing immunosuppression, EMT, and hypoxia-related pathways. These findings suggest that transcriptional reprogramming marked by differential gene expression contributes to LUAD invasiveness, and that the transition from AIS to IAC represents a critical step in early tumor progression.

SRGN-defined meta-program3 drives malignant phenotypes and immune-enriched niches in LUAD

The malignant transformation and progression of epithelial cells are key processes in LUAD development. We applied the consensus nonnegative matrix factorization (cNMF) method to decompose the full transcriptional spectrum of ITH within each tumor. This analysis identified signature modules that were further grouped into four main meta-programmes via hierarchical clustering (Fig. 3A). Gene Ontology (GO) enrichment analysis of the overrepresented genes in each transcriptional program revealed distinct biological functions. Meta-Program1 (MP1: *EMP2*, *LAMA3*, *AGER*) was enriched

in pathways related to protein secretion, the late estrogen response, and myogenesis. Meta-Program2 (MP2: *C9orf24*, *AGBL4*, *CAPS*) is associated with the DNA damage response, bile acid metabolism, and peroxisome function. Meta-Program3 (MP3: *SRGN*, *FCN1*, *VIM*) is linked to metastasis, epithelial–mesenchymal transition (EMT), and cancer cell proliferation. Meta-Program4 (MP4: *NAPSA*, *SCGB3A2*, *NEAT1*) was enriched in Myc-target gene sets, TNF- α signaling via NF- κ B, complement activation, and inflammatory responses. MP4 was additionally associated with the interferon-alpha response pathway. These results highlight the distinct functional roles of each transcriptional module in LUAD progression (Fig. 3A & Fig. S1A). These results indicate that the malignant signatures we established capture common patterns of intratumoral transcriptional heterogeneity across tumors. We subsequently analyzed the distribution of these metaprograms across pathological subtypes, revealing that MP2 was predominantly observed in normal epithelial cells, whereas MP1, MP3, and MP4 were preferentially enriched in malignant epithelial cells. (Fig. 3B & Fig. S1A). We further analyzed the copy number variations (CNVs) of these meta-programs at the single-cell level and calculated CNV scores for each (Fig. 3C). The results demonstrated that CNV scores were lowest in AAH patients and progressively increased from the AIS to the MIA and IAC, aligning with the stepwise increase in tumor malignancy during LUAD progression (Fig. 3D). Among the MP2 cells that presented the lowest CNV scores, these findings are consistent with our previous results and suggest that, while MP2s are predominantly associated with normal epithelial cells, the other MPs are more closely linked to malignant epithelial cells (Fig. 3E). We performed ssGSEA on the TCGA-LUAD dataset to quantify the expression levels of the four functional metaprogrammes and to investigate the associations between single-cell–derived functional programs and patient survival. The results revealed that MP1, MP3, and MP4 were significantly associated with poor prognosis (Fig. S1C). To further characterize the spatial distribution of these metaprograms, we projected their expression onto tissue sections. The results revealed that MP1 and MP4 were enriched primarily within tumor regions, whereas MP3 was localized predominantly at the edge of the tumor (Fig. 3F). Analysis of spatial transcriptomics data confirmed that MP1, MP3, and MP4 are predominantly enriched in cell populations located within tumor regions, suggesting that these metaprograms represent common transcriptional features associated with the tumor microenvironment. (Fig. 3G). In addition, we evaluated the spatial immune scores associated with the MP3 metaprogram and found a significant positive correlation between the MP3 score and the immune infiltration score. These findings suggest that spots with high

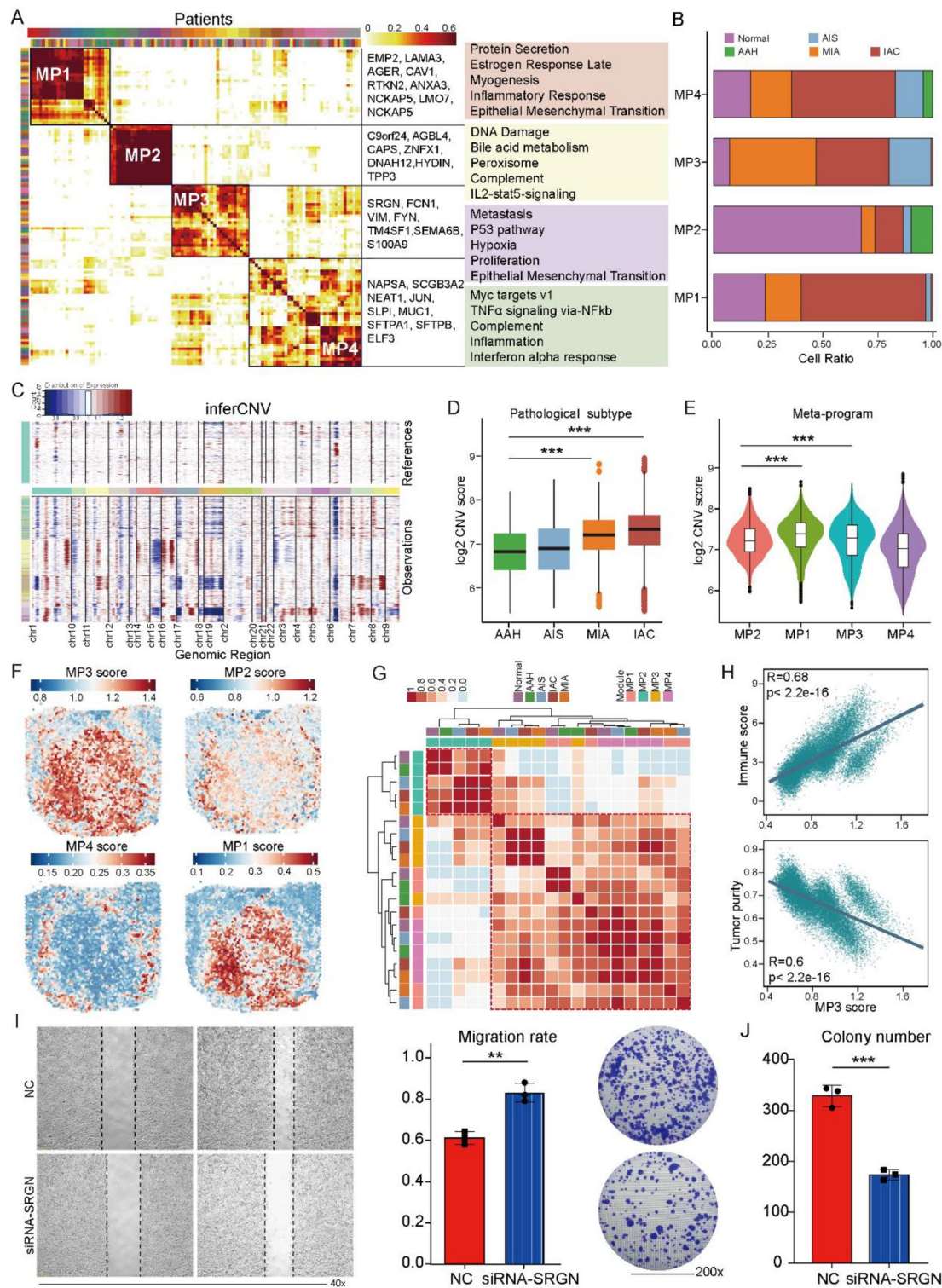


Fig. 3 (See legend on next page.)

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Fig. 3 SRGN-defined Meta-Program3 drives malignant phenotypes and immune-enriched niches in lung adenocarcinoma. **(A)** Heatmap depicting the correlation of the intratumoral modules derived from tumors. Hierarchical clustering identifies four main meta-modules. The signature genes for the four meta-modules are displayed on the right of the heatmap. **(B)** Proportion of module cells in early LUAD and advanced LUAD. **(C)** Heatmap showing large-scale CNVs in epithelial cells from 15 representative LUAD samples. The normalized CNV levels are shown; red represents a high CNV level, and blue represents a low CNV level. **(D)** Violin plots showing distributions of CNV scores among different modules from epithelial cells. **(E)** Violin plots showing distributions of CNV scores among different modules from epithelial cells. **(F)** Spatial distribution of transcriptional metaprograms. **(G)** Heatmap depicting ssGSEA scores of five functional meta-modules in patients. **(H)** Scatter plots showing the correlation between MP3 scores and immune infiltration scores. **(I)** SRGN knockdown inhibited both lung cancer cell migration and invasion in vitro. **(J)** The migration ability of the cells was assessed via a wound healing assay. The healing area of the wounds was quantified by ImageJ software 24 h after wound formation

MP3 expression are also enriched in immune cells (Fig. 3H). Previous studies have suggested that *SRGN* plays a pivotal role in tumor-stromal interaction and promotes the reprogramming of the tumor microenvironment toward a more aggressive and immunosuppressive state in LUAD [41, 42]. A549 cells were selected for functional validation experiments. Total RNA was extracted to confirm *SRGN* expression levels (Fig. S1E). The results of the CCK-8 assay revealed that, compared with the negative control, *SRGN* knockdown (si-*SRGN* group) significantly reduced cell proliferation starting at 48 h ($p < 0.01$), indicating that *SRGN* promotes tumor cell growth (Fig. S1G). Scratch wound healing assays demonstrated that *SRGN* enhances the migratory capacity of lung cancer cells ($p < 0.01$ for A549 cells). In contrast, *SRGN* inhibition significantly delayed wound closure in A549 cells ($p < 0.01$), suggesting impaired migration (Fig. 3I). Furthermore, transwell assays revealed that *SRGN* knockdown markedly suppressed both the migration and invasion abilities of A549 cells (Fig. 3J). Collectively, these findings reveal that distinct transcriptional metaprograms, particularly MP3 driven by *SRGN*, play crucial roles in promoting LUAD progression through enhancing tumor cell proliferation, migration, and invasion and shaping the immune microenvironment.

C1QC+ TAMs exhibit the M2 phenotype and drive the EMT and hypoxia pathways in LUAD progression

Studies indicate that macrophages are actively involved in antitumor immunity. To assess macrophage diversity during LUAD progression [43, 44], We reclustered myeloid into 8 subpopulations, namely, *C1QC* + Macro, *C1DC* + cDC, *CD14* + Mono, *IL32* + Mono, *KIT* + Mast, *CD16* + Mono, *LAMP3* + cDC, and *CLEC9A* + cDC (Fig. 4A and B). We further calculated the differences in the abundances of myeloid subpopulations of cells in normal, AAH, AIS, MIA and IAC patients. The proportion of *C1QC*+ Macro cells gradually increases during the progression of LUAD. In contrast, the cellular abundance of *CD14* + monocytes and *CD16* + monocytes progressively decreased during LUAD progression (Fig. 4C). We further calculated the M1 phenotype, M2 phenotype, angiogenesis and phagocytosis gene signatures for *C1QC*+ macro cells and *CD14* + Mono and *CD16* + Mono. The results revealed that *C1QC*+ macrophages

tend to constitute more of a class of macrophages with a pro-tumoral M2-like polarization while maintaining high phagocytic activity (Fig. 4D). Gene set enrichment analysis revealed that hypoxia, glycolysis, and epithelial–mesenchymal transition (EMT) pathways were significantly enriched in *C1QC*+ macrophages, and the number of pathological subtypes associated with LUAD progression progressively increased (Fig. 4E). Next, we constructed a pseudotime developmental trajectory based on the gene expression dynamics of macrophages, focusing on those subsets that exhibited significant changes during LUAD progression. *CD14* + Mono was identified as having the lowest pseudotime value and was situated at the initial point of the developmental tree, suggesting that it could serve as the developmental origin for other subclusters (Fig. 4F). These results suggest that macrophages transition before polarizing from M1 phenotype to M2 phenotype during LUAD progression, with key pathways, including cytoplasmic translation regulation, neutrophil chemotaxis, neutrophil migration, and antigen processing and presentation of peptides, playing crucial roles in this process (Fig. 4G).

Immune landscape remodeling and CD8⁺ T cell exhaustion during LUAD progression

Next, we explored the dynamics of immune cell lineages in the TME during LUAD progression. Since T cells are the most abundant type of tumor-infiltrating lymphocyte in the TME, we recustered T cells and identified 10 subpopulations (Fig. 5A), including T helper cells, *CD8*⁺ exhausted T cells, *CD4*⁺ activated T cells, *CD8*⁺ MAIT cells, *CD8*⁺ effector T cells, *CD4*⁺ regulatory T cells (Tregs), and *CD4*⁺ naïve T cells (Fig. 5B). The proportions of *CD4*⁺ naïve cells, *CD8*⁺ exhausted cells, and *CD4*⁺ Treg cells gradually increased during the progression of LUAD, and the proportion of T helper cells decreased (Fig. 5C). We further calculated the cytotoxic and exhausted gene signatures of *CD8*⁺ T cells and NK cells from AAH, AIS, MIA, and IAC patients. Both the cytotoxic and exhausted signatures gradually increased during LUAD progression (Fig. 5D and E). This corresponds to an increase in the fractions of all *CD8*⁺ T clusters from AAH patients and gradually increases across AIS, MIA, and IAC patients. We compared gene expression differences in exhausted *CD8*⁺ cells between the

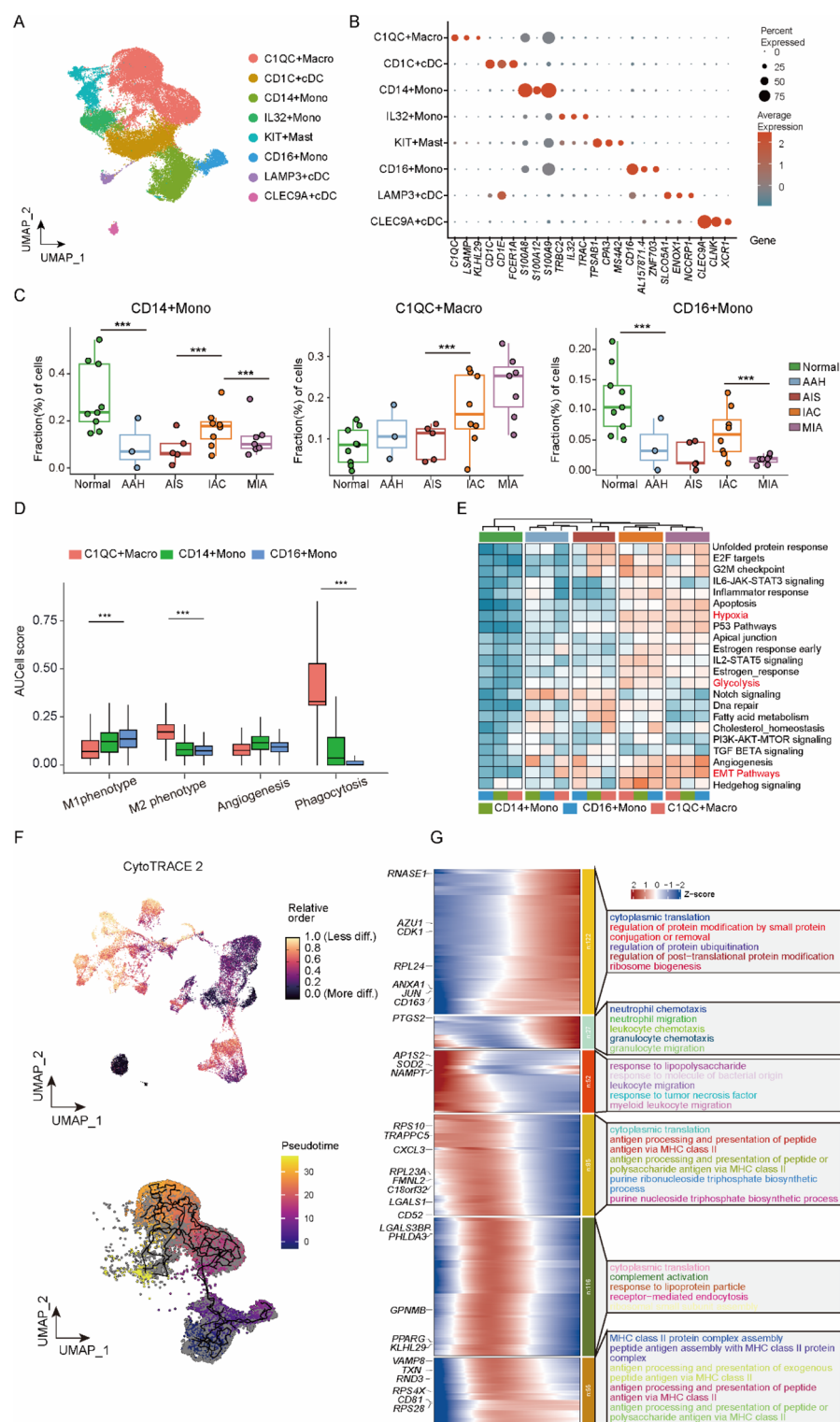


Fig. 4 (See legend on next page.)

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Fig. 4 C1QC+ TAMs exhibit the M2 phenotype and drive the EMT and hypoxia pathways in LUAD progression. **(A)** UMAP of macrophages color-coded for tissue (top) and cell type annotation. **(B)** The marker genes of the macrophage subpopulations. **(C)** Boxplots of the average number of CD14+ monocytes, C1QC+ myeloid cells, and CD16+ myeloid cells in normal, AAH, AIS, IAC, and MIA patients. The centerline indicates the median; the lower and upper hinges represent the 25th and 75th percentiles, respectively; and the whiskers denote the 1.5× interquartile range. A one-sided t test was used. All differences with adjusted $P < 0.10$ are indicated. A one-sided unpaired Wilcoxon test was used, and the P values were adjusted via the FDR method. **(D)** The GSVA scores of the M1 phenotype, M2 phenotype, angiogenesis, and phagocytosis pathways in CD14+ monocytes, C1QC+ Macro cells, and CD16+ myeloid cells. **(E)** The GSVA scores of the hallmarks of cancer in CD14+ monocytes, C1QC+ Macro cells, and CD16+ myeloid cells. **(F)** Single-cell trajectory analysis via Monocle3; cells are colored according to tissue origin and pseudotime (bottom). **(G)** Heatmaps showing gene expression dynamics during macrophage pseudotime progression

AAH and AIS groups and between the MIA and IAC groups, revealing consistent changes, indicating persistent alterations during lung cancer progression (Fig. 5F and G). We used spatial transcriptomics to map the distribution of CD8+ exhausted cells and SRGN+ malignant cells, revealing the spatial colocalization of certain cell populations (Fig. 5H). Our previous results confirmed that the colocalization of SRGN+ malignant cells and C1QC+ macrophages drives LUAD progression. Deconvolution analysis of the bulk data further revealed that in regions with high SRGN+ and C1QC+ expression, CD8+ exhausted cells were relatively abundant (Fig. 5I). The interaction between SRGN+ malignant cells and C1QC+ macrophages likely leads to the exhaustion of nearby CD8+ T cells. This interaction in the tumor microenvironment may promote immune evasion and contribute to tumor progression.

Spatial and functional crosstalk between SRGN+ malignant cells and C1QC+ macrophages drives LUAD progression

Cell signaling and communication between cancer cells and the TME are crucial for the progression and metastasis of cancer. CellChat analysis revealed the top ten interacting pathways among cells in LUAD on the basis of our scRNA-seq results (Fig. 6A). We integrated a tumor cell meta-program with previously characterized immune cell subtypes, including T cells and macrophages, to perform cell-cell communication analysis. Differential intercellular interactions were assessed across the AIS, MIA, and IAC stages using AAH as a reference. Notably, we observed progressively increased interactions between SRGN+ malignant cells and C1QC+ macrophages during LUAD progression (Fig. 6A). Next, we compared the differential receptor–ligand interaction profiles of different pathologic subtypes. The results revealed distinct receptor–ligand pairs associated with different pathological subtypes of LUAD, such as *MIF–LIFR*, *ADAM17–ERBB4*, *OSM–EGFR*, and *OSM–TNFRSF14* (Fig. 6B). To further investigate the spatial distribution patterns of key cellular interactions during LUAD progression, we leveraged spatial transcriptomics to characterize the spatial colocalization of SRGN+ malignant cells and C1QC+ macrophages (Fig. 6C). This analysis provided critical insight into the tissue-level organization of tumor-immune interactions within the

tumor microenvironment. We observed significant spatial colocalization between SRGN+ malignant cells and C1QC+ macrophages, suggesting that their proximity may facilitate intercellular interactions that contribute to LUAD progression (Fig. 6D).

Murine validation of Srgn+ malignant cells and C1QC+ macrophage interactions underlying early LUAD progression

Among various mouse strains, A/J mice exhibit the highest natural susceptibility to developing spontaneous lung tumors. To better recapitulate the initiation and progression of human lung cancer, we maintained A/J mice for 12 to 16 months. This prolonged observation period led to the emergence of three distinct types of pulmonary lesions, representing different stages of precancerous and cancerous transformation (Fig. 7A). A total of 77,107 cells from 13 samples were integrated and clustered, including 9,736 cells from 3 AAH samples, 21,563 cells from 3 adenoma samples, 27,674 cells from 4 AIS samples, and 18,134 cells from 3 normal lung samples (Fig. 7B). All the cells were annotated into 12 distinct cell types on the basis of classical marker genes, including B cells, the cycling cells, DCs, endothelial cells, epithelial cells, fibroblasts, macrophages, mast cells, mesothelial cells, monocytes, neutrophils, and T cells. To investigate the functional relevance of Srgn in lung cancer progression, we compared the gene expression profiles of Srgn-high epithelial cells and normal epithelial cells in the A/J mouse model. The analysis revealed that Srgn was coexpressed with several key genes involved in tumor initiation and progression, including *S100a9*, *S100a8*, *Il1b*, *Coro1a*, and *Stk17b* (Fig. 7C). These genes play critical roles across distinct pathological stages of lung adenocarcinoma development, such as atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ (AIS), and minimally invasive adenocarcinoma (MIA). Specifically, *S100a8* and *S100a9* are calcium-binding proteins involved in inflammatory signaling and neutrophil recruitment and are associated with early-stage lesion formation and immune microenvironment remodeling. *Il1b* encodes the proinflammatory cytokine IL-1 β , which promotes tumor-promoting inflammation and may facilitate the transition from a preneoplastic state to an invasive state. *Coro1a* is involved in actin

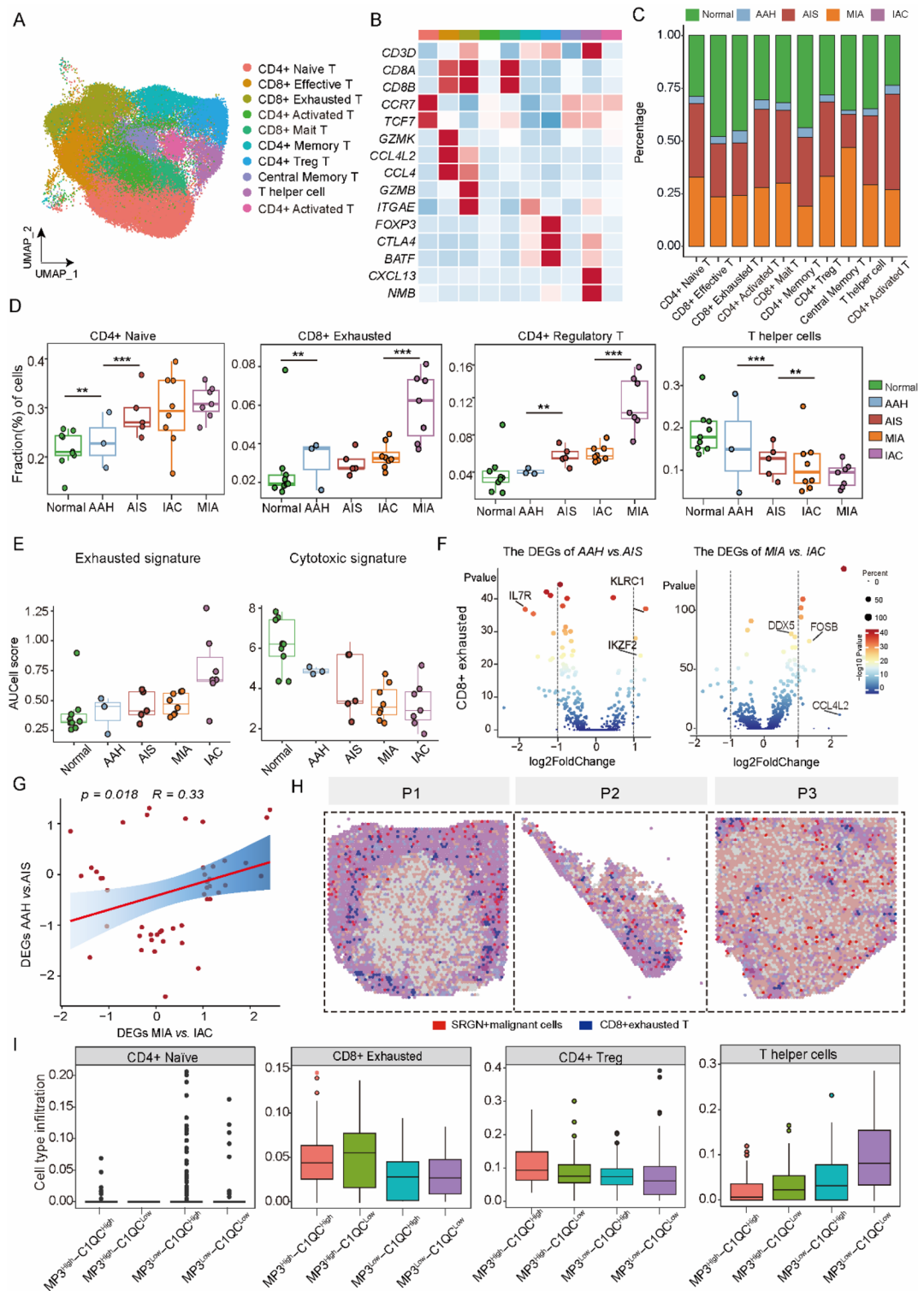


Fig. 5 (See legend on next page.)

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Fig. 5 Immune landscape remodeling and CD8+T cell exhaustion during LUAD progression. **(A)** UMAP of T cells color-coded for tissue and cell type annotation. **(B)** The heatmap illustrates marker gene expression for the major cell types. **(C)** Proportion of the subpopulations of T cells in all patients. **(D)** Boxplots of average CD4 naïve T cells, CD8+exhausted cells, and CD4+Treg cells and T helper cells in, AAH, AIS, IAC, and MIA patients. The centerline indicates the median; the lower and upper hinges represent the 25th and 75th percentiles, respectively; and the whiskers denote the 1.5× interquartile range. A one-sided t test was used. All differences with adjusted $P < 0.10$ are indicated. A one-sided unpaired Wilcoxon test was used, and the P values were adjusted via the FDR method. **(E)** The exhaustion signature and cytotoxic signature of AAH, AIS, IAC and IAC patients. **(F)** Volcano plot showing genes differentially expressed between AAH patients and AIS patients among CD8+exhausted T cells (left). The volcano plot shows genes that are differentially expressed between AAH patients and AIS patients in CD8+exhausted T cells (right). **(G)** Scatter plots showing the correlation between the DEGs of AAH vs. AIS patients and the DEGs of MIA vs. IAC patients. **(H)** Spatial distribution characterization of the subpopulations. The signature scores of SRGN+ epithelial cells and CD8+exhausted T cells in AAH, AIS and IAC LUAD patients. **(I)** Deconvolution of the proportions of CD4+ naïve, CD8+exhausted, CD4+Treg, and helper T cells across MP3 and C1QC+ macrophage-defined subgroups

cytoskeleton regulation and immune cell migration, suggesting a role in tumor–immune cell interactions. Stk17b, a serine/threonine kinase, is implicated in apoptotic regulation and may contribute to epithelial cell survival during neoplastic transformation. The co-upregulation of these genes with Srgn indicates a potential molecular network that drives the progression from preinvasive lesions (AAH and AIS) to early invasive lung adenocarcinoma (MIA), highlighting their importance as candidate targets for early intervention. Consistent with our previous analyses, the gene enrichment results revealed that Srgn-high tumor cells exhibited significant activation of pathways associated with ribosomal dysfunction, epithelial–mesenchymal transition (EMT), hypoxia, oxidative phosphorylation, and other tumor-promoting processes (Fig. 7D). Similarly, the macrophage subpopulations in A/J mice were reclassified into six distinct clusters on the basis of their gene expression profiles, with specific marker genes identified for each subset (Fig. 7E); in particular, the number of C1qc+ macrophages progressively increased across different stages of lung cancer development (Fig. 7F). Functional analysis revealed that these C1qc+ macrophages were significantly enriched in pathways associated with epithelial–mesenchymal transition (EMT), glycolysis, and DNA repair. These findings are consistent with observations from human lung cancer samples, suggesting a conserved role for C1qc+macrophages in promoting tumor progression (Fig. 7G). In addition, multiplex immunohistochemical (mIHC) staining demonstrated the close spatial proximity of Srgn+malignant cells and C1qc+macrophages in tissue sections, confirming their colocalization. Moreover, the expression of both cell types gradually increased during LUAD progression, particularly across the AAH, adenoma, and AIS LUAD subtypes (Fig. 7H). In tissue sections from A/J mice with AAH, adenoma, and AIS, cell density analysis revealed a gradual increase in C1qc⁺F4/80⁺ macrophages and SRGN⁺Nkx2-1⁺ epithelial cells during tumor progression, consistent with our previous findings (Fig. 7I). These mIHC results further support the dynamic accumulation and spatial association of Srgn+malignant cells and C1qc+macrophages during early tumor evolution. These findings suggest potential

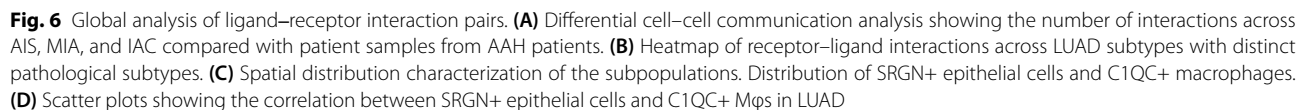
cellular interactions between these two populations that may contribute to tumor progression.

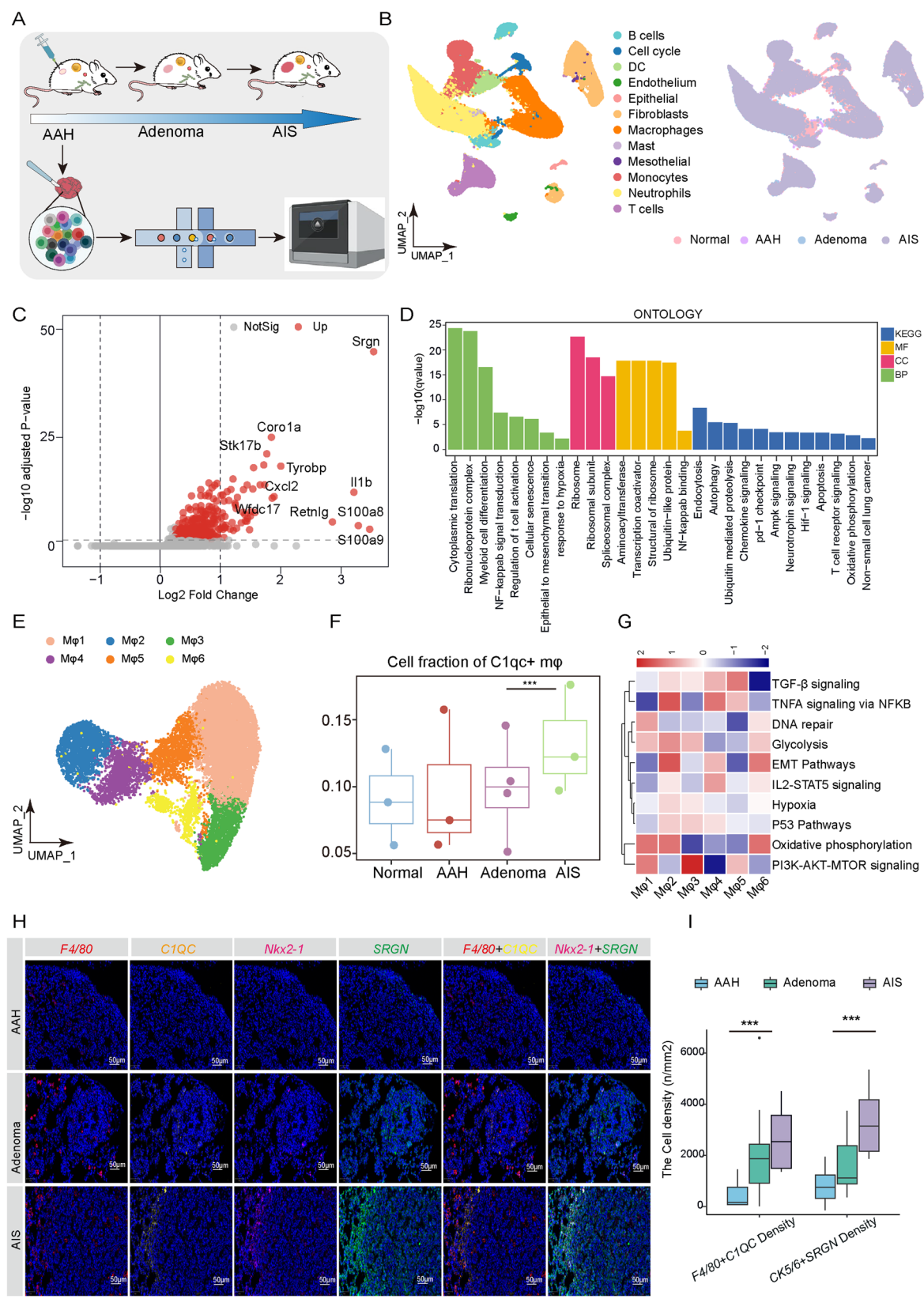
Discussion

The tumor microenvironment (TME) composed of diverse cell types, plays a critical role in tumor progression, as tumor cells themselves exhibit substantial heterogeneity [45, 46]. In this study, we present a comprehensive molecular and cellular landscape of LUAD evolution by integrating single-cell and spatial transcriptomic analyses. Through this approach, we revealed key cellular interactions that drive the early stages of LUAD progression, highlighting a cooperative malignant epithelial cell–macrophages module. Our findings emphasize the crucial role of the tumor-intrinsic epithelial program, which is characterized by high expression of *SRGN*, and the immunosuppressive function of C1QC+macrophages in shaping the TME across the early stages of LUAD progression. These results provide new insights into the mechanisms of early LUAD and suggest potential targets for early-stage therapeutic intervention.

The transition from AAH to IAC is a dynamic process characterized by gradual cellular and molecular changes that promote tumor progression [47, 48]. SRGN+malignant cells increased in abundance from AAH to IAC, paralleling the increasing presence of C1QC+macrophages in the TME. Importantly, these two celltypes show significant spatial colocalization, with the most pronounced changes in their interactions occurring at the AIS–MIA transition. This highlights the pivotal role of SRGN+malignant cells and C1QC+macrophages in facilitating tumor progression through reciprocal signaling, which promotes epithelial–mesenchymal transition and promotes a more aggressive phenotype. These findings underscore the importance of the dynamic interplay between the tumor and its microenvironment in shaping cancer progression [49, 50].

The role of C1QC+macrophages in immune suppression and tumor progression has been well documented, yet their specific involvement in early LUAD stages has not been fully elucidated [51, 52]. Our results indicate that C1QC+macrophages not only modulate the immune landscape within the TME but also actively participate





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Fig. 7 Histopathological grading and single-cell transcriptome profiles of mouse LUAD precancerous lesions. **(A)** Analysis and experimental flow chart of this study. Normal tissue and three stages of precancerous lesions (AAH, adenoma, and AIS) in A/J mice. **(B)** UMAP plot of 12 cell types from mouse scRNA-seq data. UMAP plot of different histological subtypes of the mouse scRNA-seq data. **(C)** Volcano plot of differentially expressed genes in Srgn-high epithelial cells. **(D)** Functional enrichment (GO and KEGG) of differentially expressed genes in cell populations with high Srgn expression. **(E)** UMAP plot showing macrophage subpopulations in A/J mice. **(F)** Boxplots of the average number of C1qc+Mφs in normal, AAH, adenoma, and AIS A/J mice. **(G)** Heatmap showing the pathway enrichment analysis results for the myeloid subpopulation. **(H)** Multiplex immunohistochemical (mIHC) staining of Srgn+ epithelial cells and C1qc+ macrophages in tissue sections from A/J mice with AAH, adenoma, and AIS. Scale bar, 50 μm. **(I)** Fluorescent density and proximity of C1qc+F4/80+ and Srgn+Nkx2-1+ in tissue sections from A/J mice with AAH, adenoma, and AIS

in immune evasion and tumor progression by engaging in bidirectional signaling with SRGN+malignant cells. This interplay likely contributes to the maintenance of an immunosuppressive TME, promoting the escape of tumor cells from immune surveillance and facilitating their progression toward malignancy.

Moreover, we observed that the interaction between SRGN+ malignant cells and C1QC+macrophages leads to the activation of key pathways involved in EMT, a process known to increase tumor cell migration, invasion, and metastasis. These findings are consistent with those of previous studies that highlighted the role of EMT in facilitating tumor cell plasticity and invasiveness in the LUAD. However, our study provides novel evidence linking the spatial colocalization and reciprocal signaling between SRGN+malignant cells and C1QC+macrophages as crucial mechanisms underlying EMT in early LUAD.

To further validate our findings, we used a stage-specific A/J mouse model that closely mimics human progression from AAH to IAC. This model reinforces the idea that these two cell populations play critical roles in the initiation and progression of LUAD. The use of this model also highlighted the spatial proximity of Srgn+malignant cell and C1qc+macrophages, providing strong evidence that their interactions occur within the same tumor microenvironment and contribute to tumor progression.

There are several limitations to this study. First, the analysis was based on a relatively small number of patient samples across each histological stage, which may limit the generalizability of our findings; validation in larger, independent cohorts is warranted. Second, due to technical constraints of current spatial transcriptomics platforms, spatial data were analyzed on a per-sample basis, preventing comprehensive integration of spatial patterns across all LUAD patients.

In conclusion, our study provides a detailed understanding of the molecular and cellular mechanisms driving the early stages of LUAD progression. The discovery of a cooperative SRGN+malignant cell–C1QC+macrophage module highlights a critical mechanism underlying early carcinogenesis, suggesting that interventions aimed at modulating these cell–cell interactions could serve as promising strategies for the prevention and treatment of early-stage lung cancer.

Future studies focusing on the precise molecular signaling pathways involved in these interactions and testing specific inhibitors of SRGN or C1QC+macrophages will be crucial for translating these findings into clinical applications.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12967-026-08241-8>.

Supplementary Material 1

Author contributions

Z.L. and Y.L.L. conceived the project. S.Q.M. and C.W.P. designed the experiments. S.Q.M. and C.W.P. were involved in the analysis of the experiments and wrote the manuscript. S.Q.M. and Z.Q.L. performed the bioinformatic analysis. S.Q.M. and C.W.P. and contributed to the experiments and analyzed the data. H.H. provided experimental materials. All the authors discussed the results and reviewed the manuscript.

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Data availability

All scRNA-seq data will be provided upon request to the corresponding author Li Zhang (zhangli2809@wchscu.edu.cn).

Declarations

Ethical approval

The study involving human specimens was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee on Biomedical Research, West China Hospital of Sichuan University on 18/12/2020. All animal experiments in this study were approved by the Animal Ethics Committee of the West China Hospital, Sichuan University (Approval No. 2019-195). All procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Clinical trial number: not applicable. Consent to Participate declaration: not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing of interests.

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