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Single-cell Transcriptomic Analysis Reveals an Immunosuppressive Network Between POSTN CAFs and ACKR1 ECs in TKI-resistant Lung Cancer

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Abstract. *Background/Aim: Tyrosine kinase inhibitor (TKI) therapy, a principal treatment for advanced non-small cell lung cancer (NSCLC), frequently encounters the development of drug resistance. The tumor microenvironment (TME) plays a critical role in the progression of NSCLC, yet the relationship between endothelial cells (ECs) and cancer-associated fibroblasts (CAFs) subpopulations in TKI treatment resistance remains largely unexplored. Materials and Methods: The BioProject database PRJNA591860 project was used to analyze scRNA-seq data including 49 advanced-stage NSCLC samples across three different time points: pre-targeted therapy (naïve), post-partial response (PR) to targeted therapy, and post-progressive disease (PD) stage. The data involved clustering stromal cells into multiple CAFs and ECs subpopulations. The abundance changes and functions of each cluster during TKI treatment were investigated by KEGG and GO analysis. Additionally, we identified specific transcription factors and metabolic pathways via DoRothEA and scMetabolism. Moreover, cell-cell communications between PD and PR stages were compared by CellChat. Results: ECs and CAFs were clustered and annotated using 49 scRNA-seq samples. We identified seven ECs subpopulations, with OIT3 ECs showing enrichment in the PR phase with a drug-resistance phenotype, and ACKR1 ECs being prevalent in the PD phase with enhanced cell adhesion. Similarly, CAFs were clustered into 7 subpopulations. PLA2G2A CAFs were predominant in PR, whereas POSTN CAFs were prevalent in PD, characterized by an immunomodulatory phenotype and increased collagen secretion. CellChat analysis showed that ACKR1 ECs strongly interacted with macrophage through the CD39 pathway and POSTN CAFs secreted Tenascin-C (TNC) to promote the progression of epithelial cells, primarily malignant ones, in PD. Conclusion: This study reveals that POSTN CAFs and ACKR1 ECs are associated with resistance to TKI treatment, based on single-cell sequencing.*

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Key Words: TKI treatment, POSTN CAFs, ACKR1 ECs, single cell analysis, NSCLC.

Lung cancer is the second most prevalent cancer and the leading cause of cancer-related deaths worldwide (1). The majority of patients are diagnosed at an advanced stage (2). To date, treatment of advanced lung cancer has been transformed by the discovery of oncogenic driver genes and the subsequent use of targeted therapy, particularly tyrosine kinase inhibitors (TKIs) (3, 4). However, most patients eventually develop resistance to treatment and experience disease progression (5, 6). Despite efforts, the mechanisms behind this resistance to targeted therapy remain uncertain (6, 7).

The tumor microenvironment (TME) is an important factor affecting the efficacy of TKIs (8). Previous studies revealed dynamic changes in immune cell subpopulations during TKI treatment (9). Specifically, IDO1+ macrophage, regulatory T cell and dysfunctional T cells were found to be more prevalent in PD and decreased in PR, which potentially contribute to TKI treatment resistance. However, the changes in stromal cell subpopulations during TKI treatment remain unexplored. Stromal cells encompass all cells except epithelial and immune cells, mainly comprising cancer-associated fibroblasts (CAFs) and endothelial cells (ECs) (10, 11). ECs form tumor vasculature, which provides nutrients to cancer cells and serves as a barrier to drug delivery (12, 13). Fibroblasts can enhance drug resistance in targeted therapy by secreting growth factors such as hepatocyte growth factor (HGF) (14, 15). In addition, the heterogeneity and dynamic changes of ECs and fibroblasts during targeted therapy may be pivotal in the development

of secondary drug resistance (15, 16). However, there is a lack of research exploring the dynamic changes in their heterogeneity during the TKI treatment process. Therefore, investigating the heterogeneity of these components in the TME and their evolution before and after TKI treatment will contribute to addressing the issue of secondary drug resistance in targeted therapy of lung cancer (10).

While bulk RNA-sequencing methods can process millions of cells, single-cell RNA sequencing (scRNA-seq) enables the specific profiling of cell populations at the single-cell level (10). This technique enables the characterization of stromal cell heterogeneity and the identification of dynamic phenotypic changes during treatment.

In this study, we aimed to investigate the changes occurring in stromal cells during TKI treatment in advanced lung cancer by scRNA-seq. scRNA-seq data from lung cancer patients before and after TKI treatment were obtained from the BioProject database PRJNA591860. By performing clustering analysis, we identified distinct subpopulations of ECs and CAFs that were enriched in PD or PR. Furthermore, we evaluated the cell-cell interaction between ECs, CAFs, and other type of cells during therapy process.

Materials and Methods

Data acquisition and sorting. The lung cancer single-cell sequencing data was downloaded from the BioProject database PRJNA591860 (https://www.ncbi.nlm.nih.gov/bioproject/) produced by Maynard A et al. (9). The original dataset contains the sequencing data of 49 samples (45 lung adenocarcinoma samples, 3 tumor adjacent tissue (TATs) samples, and one squamous cell carcinoma sample) with a total of 23,261 cells. The data set samples came from 30 patients, and were accompanied by detailed clinical data of the patients: patient-specific driver gene mutations, targeted therapy received by the patient, and the patient's stage in the targeted therapy process before treatment [TN, (TKI naïve)], treatment response period (*i.e.* PR after treatment), treatment resistance period (*i.e.* PD after treatment), tumor pathological stage, age, whether there was a history of smoking and other 16 indicators. The original data set also includes a preliminary clustering of all cells, dividing all cells into three clusters of epithelial cells, immune cells and stromal cells.

Data processing and unsupervised clustering. We merged gene counts as previously described (9) and extracted all stromal cells from lung adenocarcinoma samples in the dataset with R package Seurat 4.0.3. Firstly, we removed cells with less than 400 expressed genes or over 15% UMIs derived from mitochondrial genome. Genes expressed in less than three cells were also removed. After quality control, normalization was performed by dividing the UMI counts per genes by the total UMI counts in the corresponding cells and log-transforming, and scaling, centering was next conducted. Then, we performed principal component analysis (PCA) on the normalized expression matrix using highly variable genes identified by "FindVariableGenes" function and were visualized using a 2-dimensional uniform manifold approximation and projection method (Umap) or T-distributed stochastic neighbor embedding (tSNE) plot with 30 dims on the same distance metric. Finally, for the clustering of ECs, the top 15 principal components (PCs) of PCA were selected with a resolution parameter equal to 0.3 and for CAFs, the top 15 PCs were selected with a resolution parameter equal to 0.1. Analysis of epithelial cells and macrophages is the same as above.

Differentially expressed genes and cell annotation. "FindAllmarkers" function of the Seurat package was used to find the differentially expressed genes in each cell cluster. According to the identified marker genes for each cluster, we assigned them to known cell types and used the "plot_scdata" and the "plot_heatmap" function of the Scillus (Scillus, https://scillus.netlify.app/) package to visualize the unique genes of clusters. KEGG and GO analysis were performed using top 100 different expressed gene (sorted by log2 fold change) for each cluster by clusterProfiler (17).

Analysis of transcriptional factors (TFs). DoRothEA (18) is a gene set resource containing transcription factors (TFs) interacting with their targets, enabling inference of TF activity from gene expression data. The viper score of TF in cell clusters was estimated by DoRothEA within the database containing interactions with confidence level A, B and C. And "FindAllMarkers" was used to identify the top2 TFs (sorted by sorted by log2 fold change) of the clusters.

Quantification metabolism activity of each cluster. scMetabolism (19) is a R package for quantifying metabolism activity at the single-cell resolution. VISION method was used to estimate the scores of metabolism pathway and "FindAllMarkers" was used to identify the unique pathway in each cluster.

Analysis of cell-cell communication. CellChat (20) is a tool that is able to quantitatively infer and analyze intercellular communication networks from single-cell RNA-sequencing (scRNA-seq) data. Firstly, we used "filterCommunication" to filter the interaction in less than 10 cell and "computeCommunProbPathway" to estimate the communication probability based on the mRNA expression level of ligand-receptor pairs. Then, "netVisual_heatmap" and "rankNet" were used to compare the different interaction strength and pathway. Finally, we used "netVisual_bubble" to visualize the differential ligand-receptor pairs.

Statistical analysis. Gene expression comparisons among cell types and pathway comparisons between PD and PR in Cellchat were performed using unpaired two-tailed Wilcoxon rank-sum tests. All statistical analyses and presentation were performed using R 4.0.1.

Results

ECs increase in the process of TKI treatment, while CAFs decrease in PD. In order to explore the dynamic changes of ECs and CAFs populations in the process of TKI treatment, this study extracted the PRJNA591860 data set from the BioProject database, including 49 lung cancer samples from 30 patients (45 lung adenocarcinoma, 1 squamous Single-cell sequencing data of epithelial carcinoma, 3 cases of adjacent tissues). The samples were obtained from patients in three time points of therapy process: 1) before initiating systemic targeted therapy [TN, (TKI naïve)]; 2) treatment response period (*i.e.* PR after treatment) state, which includes samples taken at any

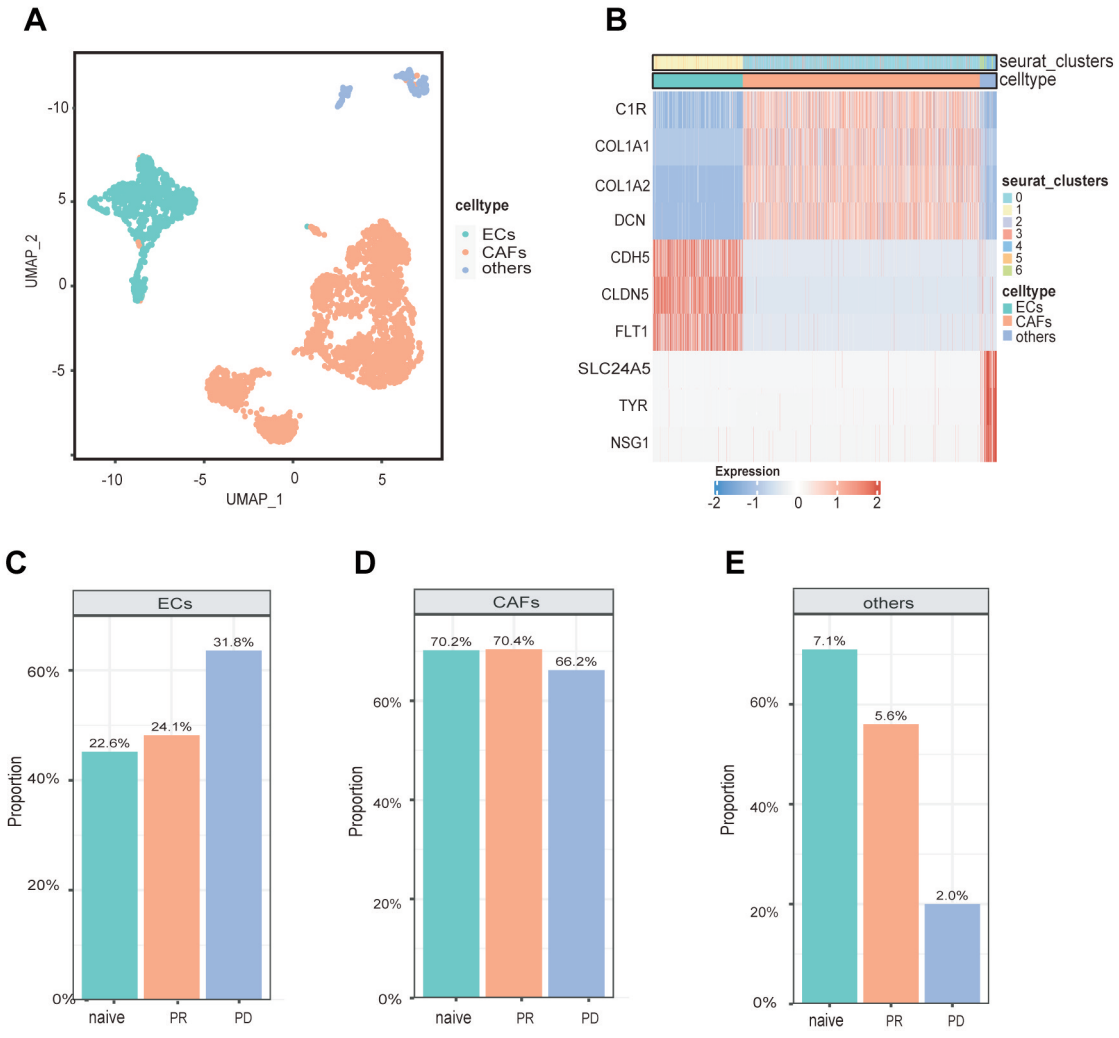

Figure 1. *Subpopulations of stromal cells at three time points. (A) Umap visualization of 3,678 stromal cells with its cluster results. (B) Heatmap showing the expression of marker genes in identified stromal cell clusters. The top bars labeled the clusters corresponding to specific cell types. (C-E) The proportion of stromal cell clusters (ECs, CAFs and others) in different periods of TKI treatment. ECs: Endothelial cells; CAFs: cancer-associated fibroblasts; PR: partial response; PD: progressive disease.*

time during treatment with targeted therapy while the tumor was regressing or stable by clinical imaging (PR) and upon subsequent progressive disease as determined by clinical imaging; 3) at the stage when tumors showed acquired drug resistance [progression (PD)] (9). The data set contains a total of 3678 stromal cells, and Principal Component Analysis (PCA), cluster analysis and Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction were applied to divide the cells into seven subpopulations.

Based on the established markers of stromal cells in lung cancer (16), seven clusters were grouped and annotated (Figure 1A). CLDN5, FLT1, CDH5 were used as EC markers, and COL1A1, DCN, COL1A2, and C1R were used as CAFs markers. We defined 979 ECs and 2659 CAFs (Figure 1B) and used a histogram to compare the proportion of these cells in different periods (Figure 1C-E). The results showed that the proportion of ECs continued to increase from TN to PD, which may provide tumor nutrition and promote tumor progression in PD (2, 21, 22). While proportion of CAFs tended to drop in PD, which illustrated that CAFs had a weak proliferative capacity in PD, but its other functions may be enhanced.

OIT3 ECs enriched in PR phase and ACKR1 ECs enriched in PD phase. To further clarify the changes of EC subsets during targeted therapy, all 979 ECs were extracted and further

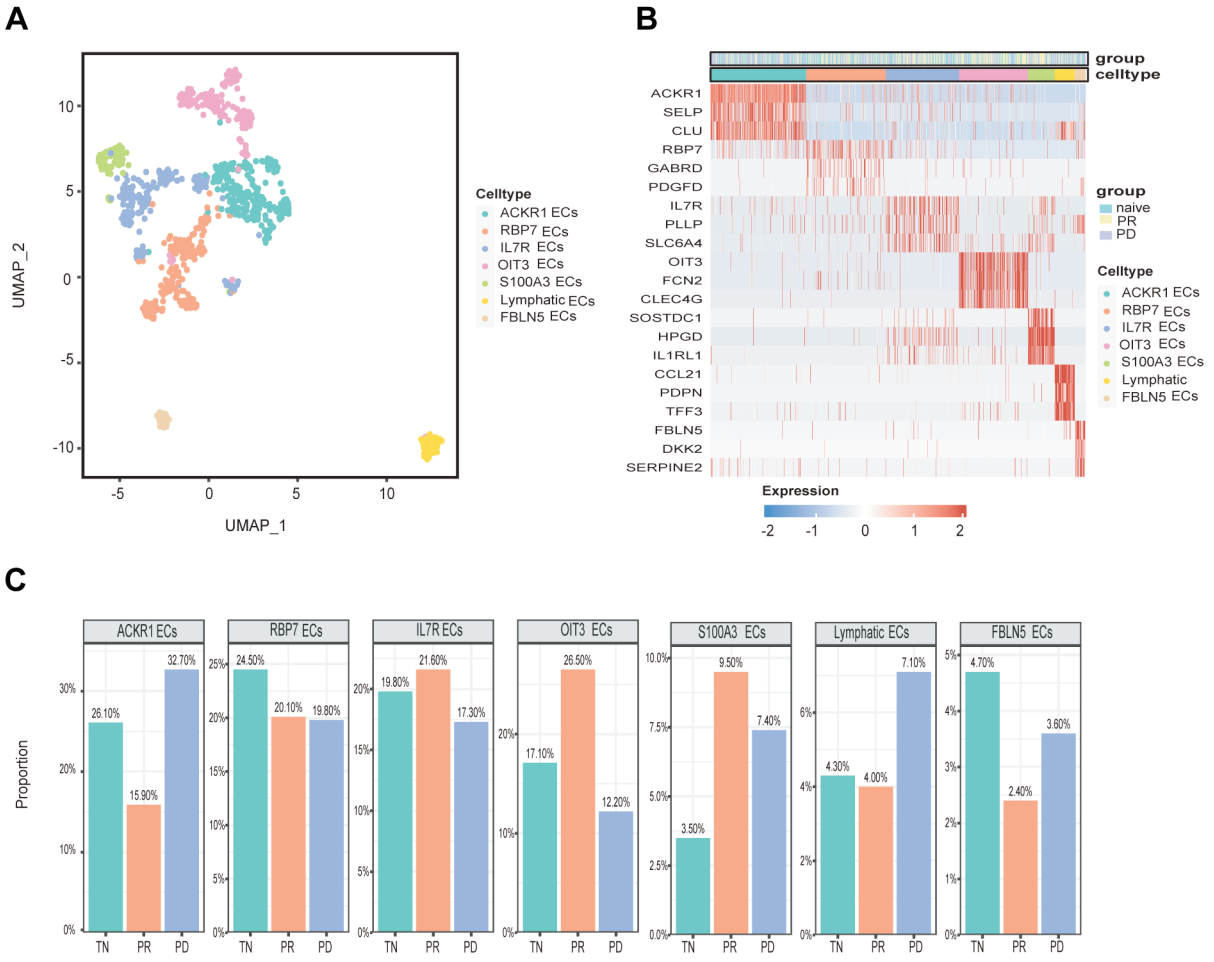

Figure 2. *ACKR1 ECs enriched in PD stage and OIT3 ECs enriched in PR stage. (A) Umap visualization of ECs subtypes derived from TN, PR and PD patients. (B) Heatmap showing the expression of marker genes in each subtype of ECs and their associated clinical stage information. (C) Fraction of cells belonging to each treatment stage for ECs clusters. ECs: Endothelial cells; PR: partial response; PD: progressive disease.*

divided into 6 clusters. We next attempted to identify marker genes for each of these clusters and to assign them to known EC subtypes (Figure 2A and B). In addition, we calculated the fraction of cells originating from each of the three treatment groups in each of the 6 EC clusters (Figure 2C).

ACKR1 and lymphatic ECs (TFF3+,PDPN+) were enriched in PD, with a strong matrix secretion(CLU) and intercellular signal transduction (ACKR1, CCL21) (Figure 2B; Figure 3A, B), associated with an phenotype of activated ECs previously describe in prostate cancer (23, 24). And ACKR1 ECs overexpressed ACKR1 and SELP, both of which play an important role in promoting the adhesion and tissue migration of immune cell (25, 26). OIT3 and S100A3 ECs were enriched in PR, expressed features associated with drug resistance-related metabolic enzymes (HPGD) and abnormal phagosome (CLEC4G) (Figure 2B; Figure 3A, B). In addition, we identified RBP7 and FBLN5 ECs preferentially enriching in TN and IL7R ECs distributing in all periods.

Finally, to further assess the difference in Transcription Factor (TF) and metabolism pathway activities among our ECs clusters, DoRothEA and scMetabolism were applied. We identified FOSL2 and JUND as candidate transcription factors underlying rapid proliferation of ARCK1 ECs as previously described (27, 28). Moreover, elevated Glycolysis and Pyrimidine metabolism reflects its activation phenotype, which may be was one of the efficient target of ACKR1 ECs to inhibit angiogenesis in PD (29, 30) (Figure 3C, D). Likewise, in OIT3 ECs, genes regulated by NR2F2 and HHEX were highly upregulated and Riboflavin metabolism were activated, which may contribute to its resistant phenotype (31).

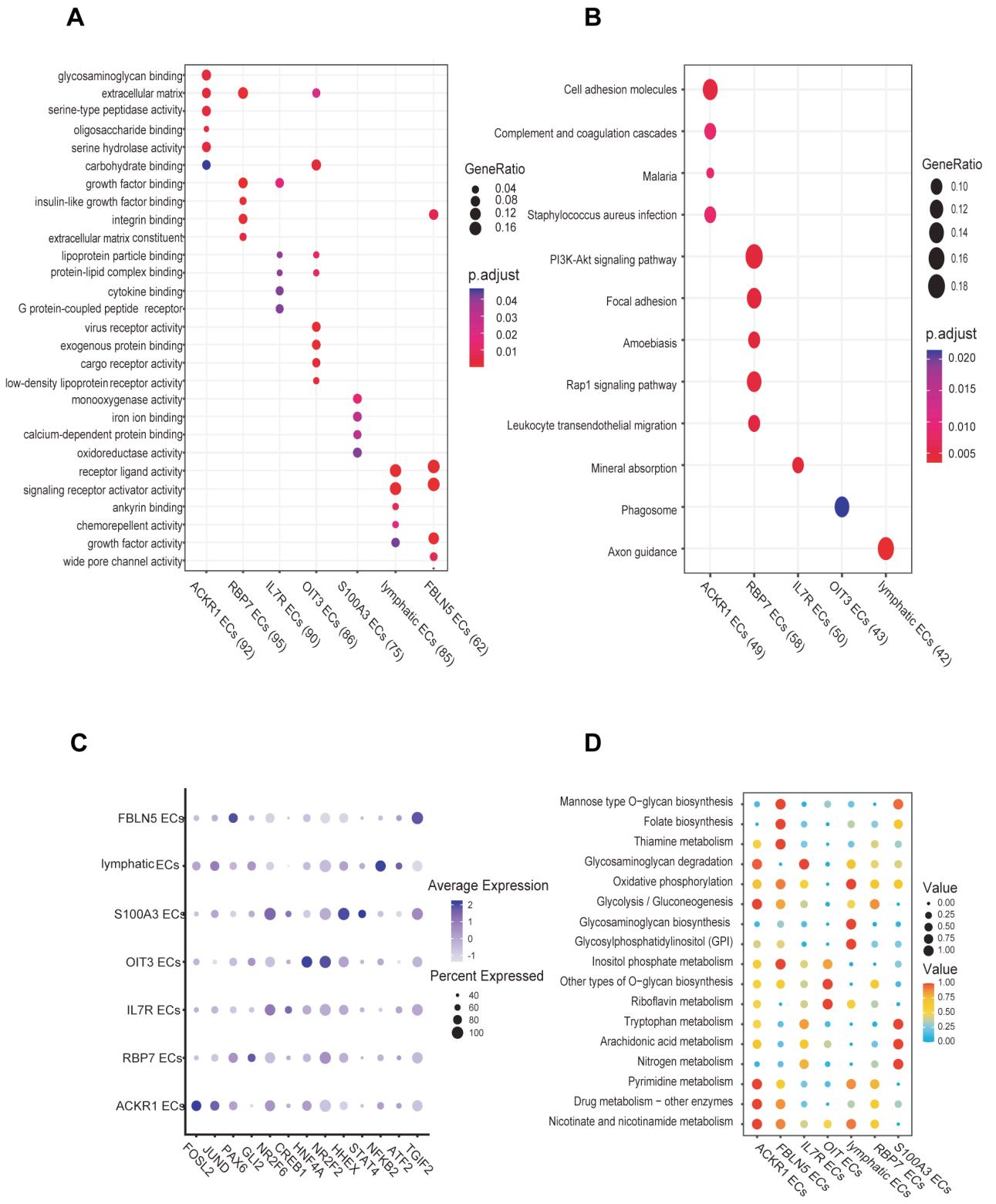

Figure 3. *Different EC clusters present distinct functions and metabolisms. (A-B) GO and KEGG enrichment result for the top 100 markers of each ECs cluster. In parentheses is the number of genes matched to the pathways. (C) Bubble chart of the estimated regulon activity of transcription factors in each ECs cluster. (D) Bubble chart showing the metabolism pathways enrichment result calculated by scMetabolism. ECs: Endothelial cells; PR: partial response; PD: progressive disease.*

ACKR1 ECs mediate macrophage function through the CD39 signaling pathway in PD. With the dynamic change of EC's phenotype across three treatment periods, we hypothesized that ECs also have distinctive interactions with other cells in different periods. To explore the cell-cell interaction between ECs clusters and other cell types, we performed CellChat analysis and found ECs had a strong interaction strength with macrophage (MF) (Figure 4A). Next, we re-clustered, annotated the macrophage and compared the macrophage-ECs interactions between PR and PD and observed that ACKR1 ECs - SLC2A5 MF interactions were enhanced while OIT3 ECs - SLC2A5 MF and OIT3 ECs - EGR1 MF were decreased in PD (Figure 4B). These collective findings suggested that different ECs subtypes had distinctive interactions with macrophages and some interactions may promote drug resistance from unique cell cluster.

To further explore the ACKR1 ECs - macrophage communication pathways in PD, we compared the informative flow between PR and PD and found that CD39 related pathway were enriched in PD (Figure 4B), which can converse extracellular ATP (eATP) to AMP and thus potentially inhibit eATP-P2-mediated proinflammatory response (32). We also verified the expression of ENTPD1 (encoding CD39) in ECs during the treatment and found that its expression decreased in PR but increased in PD and expressed mainly in ACKR1 ECs, which further proved the correlation between ECs CD39 pathway activation and PD period (Figure 4C).

In summary, ACKR1 ECs presented a unique interaction with macrophage. It can promote macrophage migration through ACKR1 and SELP and activate the CD39 pathway to inhibit macrophage function and may promote targeted therapy resistance. Thus, our result suggested that targeting CD39 combined with TKI therapy may prolong the efficacy of TKI treatment which need further verification by experiments.

POSTN CAFs enriched in PD phase and PLA2G2A CAFs enriched in PR. To further clarify the changes of CAFs subsets during TKI treatment, all 2659 CAFs were extracted and further divided into 7 clusters. Then, we attempted to identify marker genes for each of these clusters and assigned them to known CAFs subtypes (Figure 4A, B). In addition, we calculated the fraction of cell clusters from each of the three treatment phases (Figure 5C).

POSTN CAFs enriched in PD, with a strong matrix secretion (COL10A1) and intercellular signal transduction function (CTHPC1, SULF1) (Figure 5B; Figure 6A, B). POSTN CAFs overexpressed CTHPC1, COL10A1, POSTN. According to previous studies, the CTHPC1 gene and its products can interact with the Wnt pathway and TGF-β/Smad pathway to promote the migration of tumor cells (33, 34). COL10A1 has been shown to increase expression in gastric cancer tissues and plays an important role in promoting epithelial-mesenchymal transition (EMT) (35, 36). The protein encoded by POSTN can supports the adhesion and migration of epithelial cells, promoting cancer stem cell maintenance and metastasis (37). Therefore, we believed that POSTN CAFs were closely related to tumor metastasis.

PLA2G2 CAFs enriched in PR, with a strong intercellular signal transduction and immune activity (Figure 5B; Figure 6A, B). PLA2G2 CAFs overexpressed SCARA5 and PLA2G2A. Previous studies have suggested that SCARA5 acts as a tumor suppressor gene, which mediates G_2/M cell cycle arrest by inhibiting FOXM1 expression and inhibits the expression of G_2/M cyclins and kinases in A549 cells (38). Most of the known functions of PLA2G2A are related to inflammation, immune response, antithrombosis, cell proliferation, ischemic injury and allergy (39). Studies in both ovarian and gastric cancer have shown that PLA2G2A can inhibit the proliferation, invasion and migration of tumor cells by acting on β-catenin (40). Therefore, we believe that PLA2G2A may play a role in inhibiting tumor progression during PD.

Finally, Dorothea and scMetabolism were used to assess the difference in Transcription Factor (TF) and metabolism pathway activities among CAFs clusters (Figure 6C, D). POSTN CAFs was highly enriched at transcription factors HHEX and ZEB2 while the expression levels of ZEB1 and NR3CI were higher in PLA2G2A CAFs. Both POSTN CAFs and PLA2G2A CAFs are metabolically active.

POSTN CAFs mediate epithelial cells, growth through the TENASCIN signaling pathway in PD. To dissert the dynamic interaction between CAFs and other cells, we performed CellChat analysis and found CAFs had a strong interaction with epithelial cells (mainly malignant cells) (Figure 7A). We reclustered, annotated the epithelial cells, compared the epi-CAFs interactions between PR and PD and found that POSTN CAFs – KRT7 epi interactions were sharply enhanced in PD while FGFR4 interacts with KRT7 in opposite trends (Figure 7B). These collective findings suggested that different CAFs subtypes had distinctive interactions with epithelial cells, consistent to its great heterogeneity. And the enhanced POSTN CAFs – KRT7 epi interaction may contribute to the tumor progression in PD.

To further explore the POSTN CAFs - epithelial cells communication pathways in PD, we compared the informative flow between PR and PD and found that TENASCIN pathway was significantly enriched in PD (Figure 7C), which can promote the epithelial-mesenchymal transition and cell proliferation of cancer through TNC-integrins interaction as previously describe (41). We next verified the TNC expression in CAFs and ITGAV expression in epithelial cells found them mainly expressed in PD (Figure 7D, E), which further proved the activation of TENASCIN pathway in PD phase.

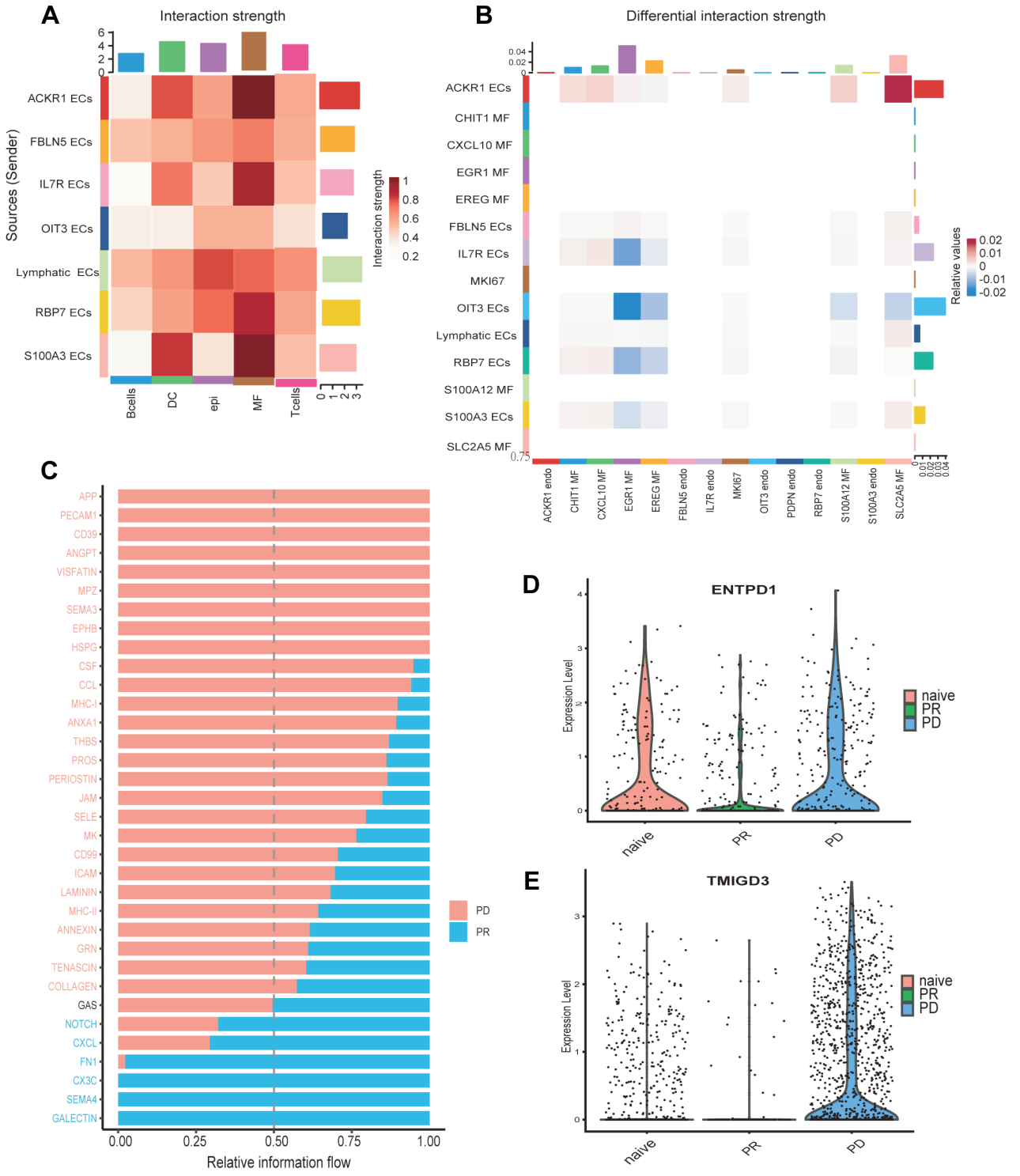

Figure 4. *CellChat analysis reveals CD39 related MF- ACKR1 ECs interactions enriched in PD. (A) Heatmap showing the cell-cell communication strength between ECs clusters and other cell types. (B) Heatmap of differential MF- ECs cell interaction between PR and PD. The elevated interaction (red block) and down-regulation (blue block) interaction in PD is shown. (C) Histogram showing the up-regulated MF-ACKR1 ECs interaction pathway in PD. The score of informative flow was calculated by summing all the interaction probability in PD and PR. The pathway significantly enriched in PD is indicated with red letters, while the pathway significantly enriched in PR is indicated with blue letters, p<0.01. (D) Violin plot illustrating the expression of ENTPD1 of ECs at different treatment time points. (E) Violin plot indicating the expression of TMIGD3 of macrophage in different treatment time points. ECs: Endothelial cells; PR: partial response; PD: progressive disease.*

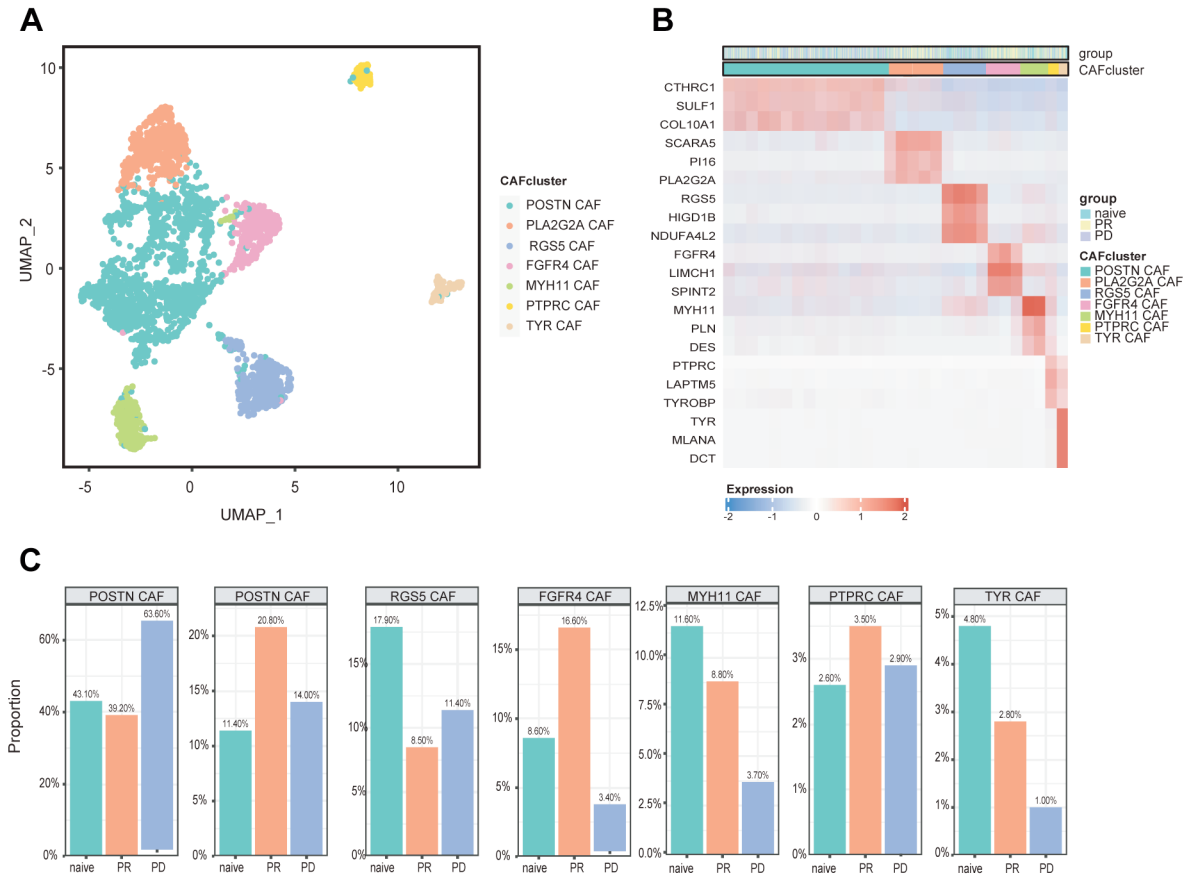

Figure 5. *POSTN CAFs enriched in PD phase and FGFR4 CAFs, PLA2G2A CAFs enriched in PR. (A) Umap visualization of fibroblast subtypes derived from TN, PR, and PD patients. (B) Heatmap showing the expression of marker genes in each subtype of CAFs. (C) Fraction of cells belonging to each treatment stage for each CAFs cluster. CAFs: Cancer-associated fibroblasts; PR: partial response; PD: progressive disease.*

In summary, POSTN CAFs had a unique interaction with epithelial cells. It can promote the tumor epithelial-mesenchymal transition (EMT) and invasion through secreting CTHPC1, POSTN and TNC-integrins ligand-receptor pairs. Thus, our result suggested that targeting POSTN CAFs combined with TKI therapy may enhance the efficacy of TKI treatment through reducing synthesis of abnormal matrix secreted proteins.

Discussion

Targeted therapies against oncogene-driven cancers are frequently employed, which target the corresponding oncoproteins. However, these treatments often result in incomplete tumor response, followed by regrowth due to the acquisition of drug resistance. The mechanisms underlying resistance to targeted therapy remain poorly understood. In our study, we utilized single-cell sequencing data from patients with advanced-stage NSCLC before and after targeted therapy. Our analysis identified ACKR1 ECs and POSTN CAFs as enriched in the PD stage. We discovered that ACKR1 ECs interact with macrophages and suppress their function through the CD39 pathway. Additionally, POSTN derived TNC produced by CAFs may facilitate tumor EMT and enhanced invasion, both of which contribute to TKI treatment resistance. Through our analysis at various treatment time points, we revealed substantial muti-omics diversity within stromal cells and observed dynamic changes in cell phenotypes and cell-cell interaction during treatment. These findings present novel insights for enhancing the efficacy of targeted therapy in advanced NSCLC.

The concurrent use of vascular endothelial growth factor (VEGF) inhibitors and EGFR-TKI has emerged as a promising therapeutic strategy in advanced-stage NSCLC (42, 43), significantly extending progression-free survival and delaying TKI resistance, as evidenced in multiple clinical trials (44, 45). However, the exact mechanism by which antiangiogenic therapy impedes the development of resistance to targeted treatments

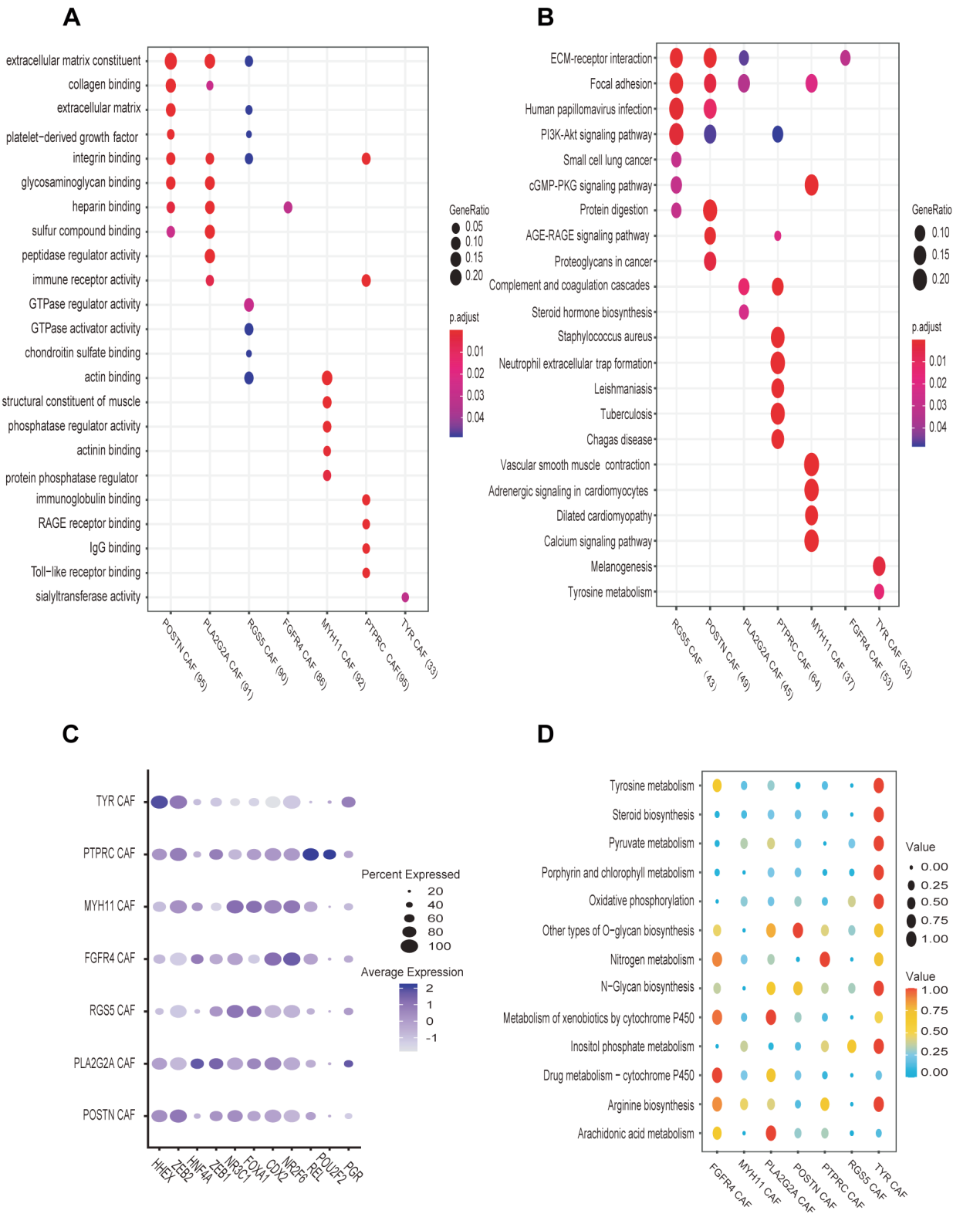

Figure 6. *Different CAFs clusters present distinct functions and metabolisms. (A-B) GO and KEGG enrichment result for the top 100 markers of each CAFs cluster. In parentheses is the number of genes matched to the pathways. (C) Bubble chart of the estimated regulon activity of transcription factors in each CAFs cluster (D) Bubble chart showing the metabolism pathways enrichment calculated by scMetabolism. CAFs: Cancer-associated fibroblasts; PR: partial response; PD: progressive disease.*

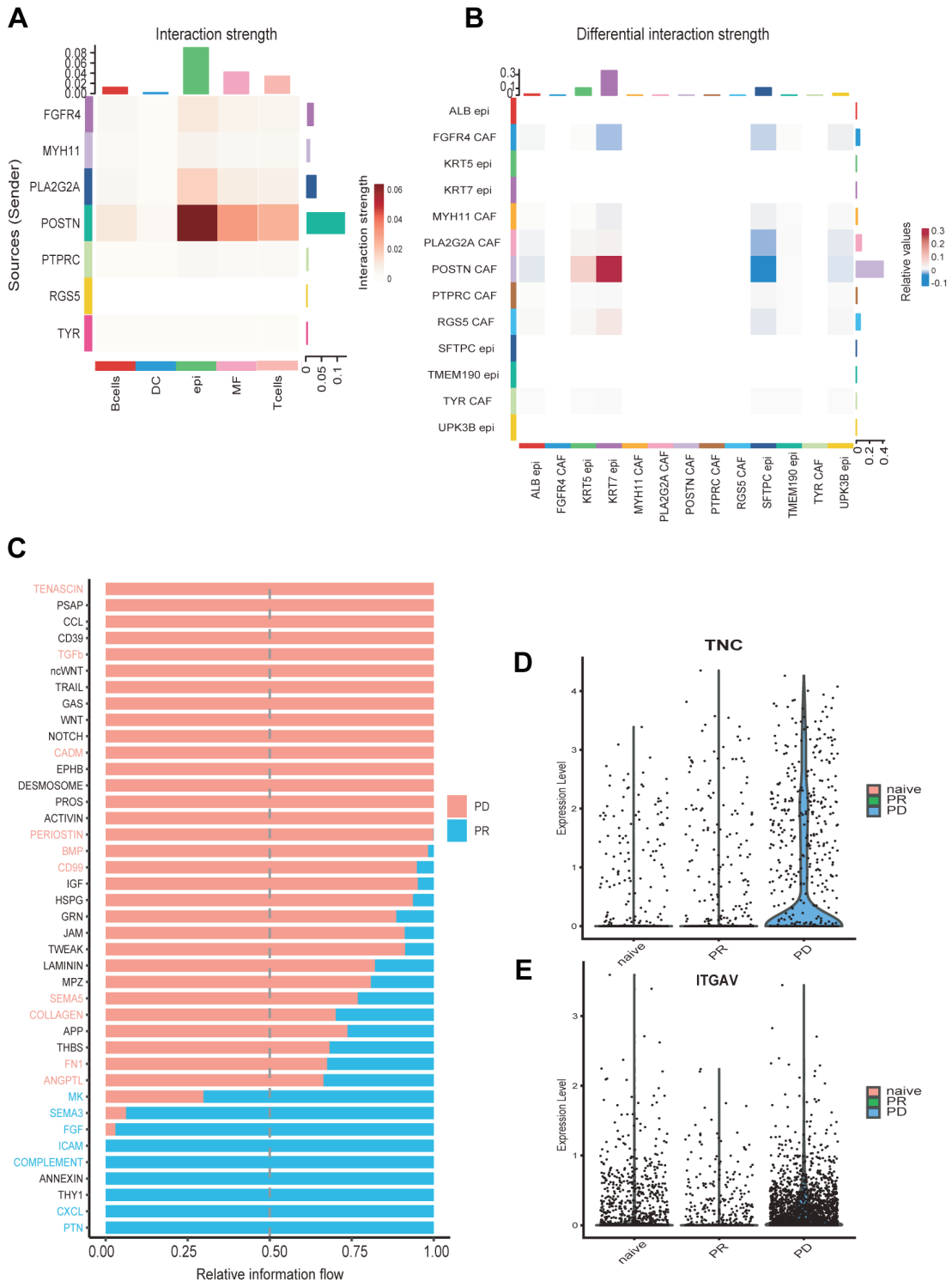

Figure 7. *CellChat analysis reveals TENASCIN related epi-CAFs interactions enriched in PD. (A) Heatmap showing the cell-cell communication strength between CAFs clusters and other cell types. (B) Heatmap of differential epi-CAFs cell interaction between PR and PD. The elevated interaction (red block) and down-regulation (blue block) interaction in PD is shown. (C) Histogram showing the up-regulated epi- POSTN CAFs interaction pathway in PD. The score of informative flow was calculated by summing all the interaction probability in PD and PR. The pathway significantly enriched in PD is indicated with red letters, while the pathway significantly enriched in PR is indicated with blue letters, p<0.01. (D) Violin plot illustrating the expression of TCN of CAFs in different treatment time points. (E) Violin plot showing the expression of ITGAV of epithelial cells in different treatment time points. CAFs: Cancer-associated fibroblasts; PR: partial response; PD: progressive disease.*

remains elusive. Here, we found ACKR1 ECs enriched in the PD phase of TKI therapy, characterized by an upregulated ECM–receptor interaction, enhanced focal adhesion, and active immune cell engagement, akin to previously observed activated ECs in prostate, colorectal and ovary cancers (23). Conversely, OIT3 ECs, predominating in the PR phase and exhibiting FN2 over-expression, can inhibit metastasis and EMT in HCC (46), indicating their potential role in augmenting TKI efficacy *via* the TGFβ/FN2/EMT pathway. Further exploration of their phenotype revealed a robust interaction with macrophages *via* the CD39 pathway in PD. CD39, a leukocyte and endothelial cell plasmalemma enzyme, mitigates platelet activation and leukocyte infiltration by hydrolyzing ATP/ADP (47). ACKR1 ECs may prompt macrophages to adopt an immunosuppressive phenotype through high expression of CD39, thereby fostering TKI treatment resistance. Conversely, anti-CD39 could bolster human T-cell proliferation, attenuate macrophage activity, and curb tumor growth, particularly when combined with anti-PD1 therapy (32). These insights underscore CD39's potential as a therapeutic target to enhance TKI responses by revitalizing the immune response in PD.

CAFs present a tempting and promising therapeutic target for cancer intervention, however, the effectiveness of CAFs targeting in enhancing targeted therapy remains incompletely understood (48). Here, we identified POSTN CAFs enriches in PD, which secrete extensive amounts of extracellular matrix proteins and promotes tumor EMT, similar to previously described CAFs in gastric (49) and lung cancers (16). This suggests that POSTN CAFs may contribute to TKI resistance *via* the EMT pathway, making their depletion a potential strategy to improve the efficacy of TKI treatment. In addition, we discovered PLA2G2A CAFs and FGFR4 CAFs enriched in PR. Notably, FGFR4 CAFs, which overexpress LIMCH1, suppress the growth of lung cancer by interacting with HUWE1 (50), hinting they could promote the efficacy of TKI treatment. Furthermore, we demonstrated that POSTN CAFs intensively interact with epithelial cells through secreting Tenascin-C, which binds to various receptors, including integrin, receptor protein-tyrosine phosphatase-ζ/β and EGF receptor (41). This interaction promotes EMT *via* FAK phosphorylation, as observed in breast cancer (51). Therefore, targeting Tenascin-C represents a promising approach to hinder tumor progression and extend the efficiency of TKI treatments, which have shown survival benefits when combined with chemoradiotherapy in malignant gliomas (52).

Conclusion

Our study, based on single-cell sequencing, uncovered the association of POSTN CAFs and ACKR1 ECs with resistance to TKI treatment. This revelation provides new insights into potential directions for targeting stomal cells to enhance the durability of TKI response in advanced NSCLC.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available in the [BioProject] repository [https://www.ncbi.nlm.nih.gov/bioproject/]

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

CCH designed the study. WZY, YN and SHL performed the data analyze and figures generation. SJY and XYZ drafted, edited, and revised the manuscript. All Authors have written, reviewed and approved the manuscript.

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