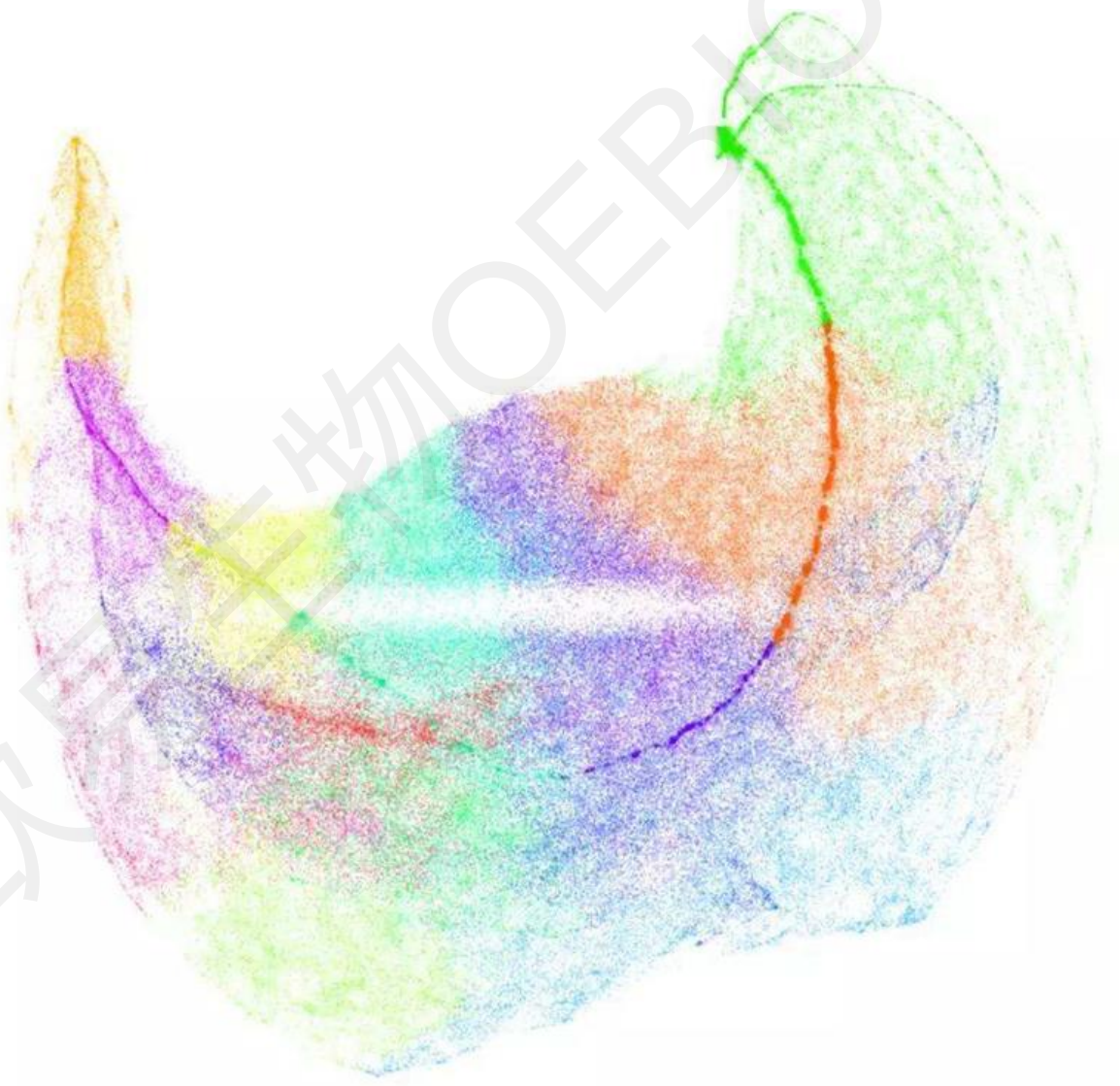
欧易生物

单细胞文献集



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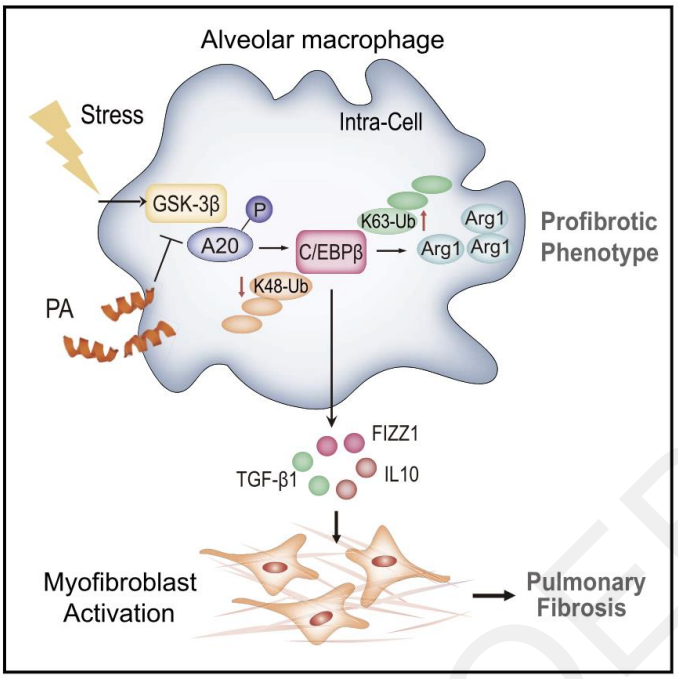
Article

Immunity

Targeting Degradation of the Transcription Factor C/EBPβ Reduces Lung Fibrosis by Restoring Activity of the Ubiquitin-Editing Enzyme A20 in Macrophages

Graphical Abstract



Authors

Shan-shan Liu, Xiao-xi Lv, Chang Liu, ..., Xia Li, Bo Huang, Zhuo-wei Hu

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In Brief

Dysregulation of the ubiquitin-editing enzyme A20 contributes to the development of several human inflammatory diseases. Liu et al. demonstrate that suppression of A20 enzymatic activity in alveolar macrophages promotes lung fibrosis by reducing transcriptional factor C/EBPβ degradation and enhancing targeted gene expression, which directs the profibrotic phenotype of macrophages.

SUMMARY

Although recent progress provides mechanistic in- sights into the pathogenesis of pulmonary fibrosis (PF), rare anti-PF therapeutics show definitive promise for treating this disease. Repeated lung epithelial injury results in injury-repairing response and inflammation, which drive the development of PF. Here, we report that chronic lung injury inactivated the ubiquitin-editing enzyme A20, causing progressive accumulation of the transcription factor C/EBPb in alveolar macrophages (AMs) from PF patients and mice, which upregulated a number of immunosuppressive and profibrotic factors promoting PF development. In response to chronic lung injury, elevated glycogen synthase kinase-3b (GSK-3b) interacted with and phosphorylated A20 to suppress C/EBPb degradation. Ectopic expression of A20 or pharmacological restoration of A20 activity by disturbing the A20-GSK-3b interaction accelerated C/EBPb degradation and showed potent therapeutic efficacy against experimental PF. Our study indicates that a regulatory mechanism of the GSK-3b-A20-C/EBPb axis in AMs may be a potential target for treating PF and fibroproliferative lung diseases.

Zhou *et al. Cancer Cell Int* (2019) 19:190
https://doi.org/10.1186/s12935-019-0911-1

Cancer Cell International

PRIMARY RESEARCH Open Access

Characteristics of a novel cell line ZJU-0430 established from human gallbladder carcinoma

Fei Zhou[1], Yanhua Zhang[2*], Jihong Sun[1*] and Xiaoming Yang[1,3*]

Abstract

Background: Gallbladder cancer is the most common malignant neoplasm of the biliary tract, responsible for 80–95% of cases. Appropriate models are required for investigating the molecular pathogenesis of gallbladder cancer.

Methods: In this study, we aimed to establish a gallbladder cancer cell line from primary tumour. Single cell RNA sequencing, Light and electron microscopy, DNA content analysis, cytogenetic analysis, short tandem repeat (STR) DNA fingerprint analysis, immunophenotypic characterization, and xeno-transplantation were utilized to characterize the novel ZJU-0430 cell line in vitro and in vivo.

Results: The cell line showed multiple cell shapes and characteristic epithelial morphologies under the microscope, but no too much heterogeneity by scRNA-Seq, with a population doubling time (PDT) of 19.81 h, which was shorter than that for GBC-SD cells. An immunophenotypic analysis revealed that ZJU-0430 cells were positive for CD24, CD44, CD29 and CD133 expression, and partially positive for CD184, and CD326 expression, and negative for CD34, CD90, CD117, and CD338 expression, similar to the primary cancer cells. A pathological analysis confirmed the origination of cell line from gallbladder tumour. ZJU-0430 cells had higher migration, invasion and proliferation properties than GBC-SD cells in vitro, and showed in vivo tumorigenicity in nude mouse xenograft settings.

Conclusions: The results confirm the potential utility of ZJU-0430 cell line as a representative model of gallbladder cancer and suggest that it could be used in the in vitro and in vivo studies of gallbladder cancer pathogenesis and to develop new therapeutics.

Keywords: Gallbladder cancer, ZJU-0430 cell line, Short tandem repeat, Epithelial, Karyotype analysis, Tumorigenicity

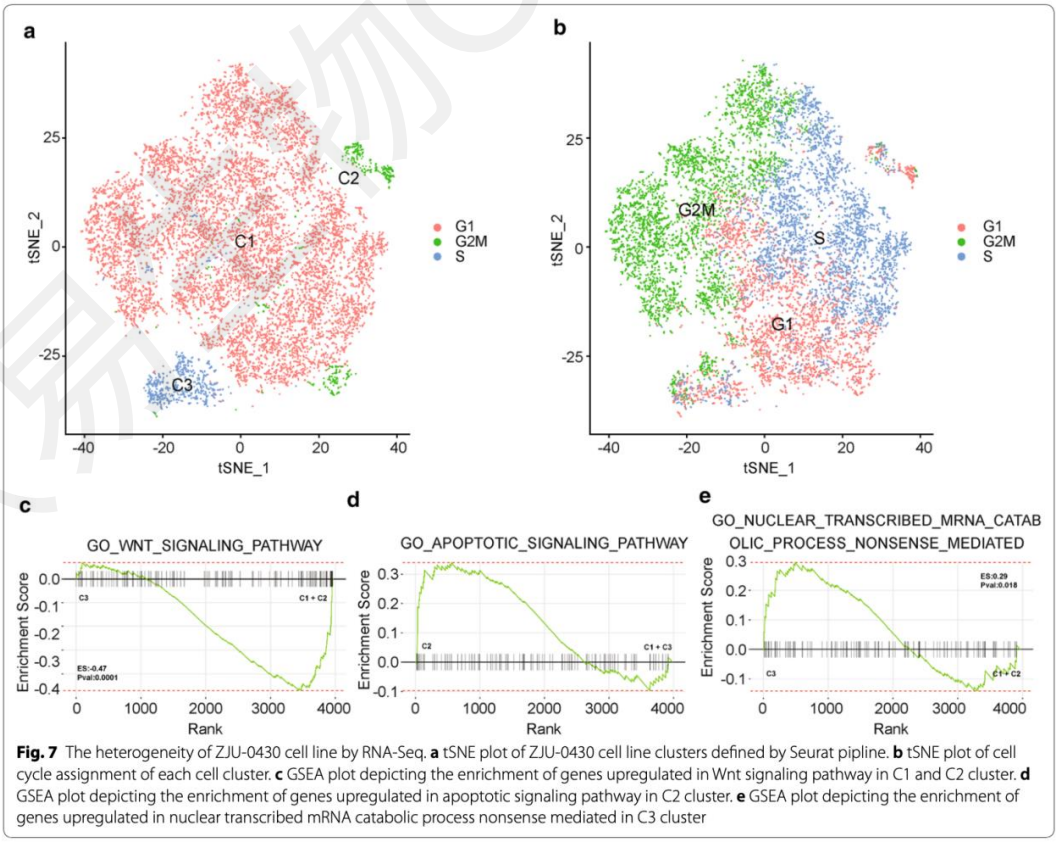

Fig. 7 The heterogeneity of ZJU-0430 cell line by RNA-Seq. **a** tSNE plot of ZJU-0430 cell line clusters defined by Seurat pipline. **b** tSNE plot of cell cycle assignment of each cell cluster. **c** GSEA plot depicting the enrichment of genes upregulated in Wnt signaling pathway in C1 and C2 cluster. **d** GSEA plot depicting the enrichment of genes upregulated in apoptotic signaling pathway in C2 cluster. **e** GSEA plot depicting the enrichment of genes upregulated in nuclear transcribed mRNA catabolic process nonsense mediated in C3 cluster

FULL PAPER

ADVANCED FUNCTIONAL MATERIALS
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Combinatorial Photothermal 3D-Printing Scaffold and Checkpoint Blockade Inhibits Growth/Metastasis of Breast Cancer to Bone and Accelerates Osteogenesis

Chao He, Luodan Yu, Heliang Yao, Yu Chen,* and Yongqiang Hao**

Cancer metastases are the main causes for the high mortality of cancer. The current treatment modality for bone metastasis of breast cancer is dominantly destructive, which urges the engineering of multifunctional biomaterials, not only for eliminating primary/metastases tumors effectively but also for enhancing bone–tissue regeneration. Herein, an immune adjuvant (R837)-loaded and niobium carbide (Nb_2C) MXene-modified 3D-printing biodegradable scaffold (BG@NbSiR) is designed and constructed to effectively treat bone metastasis of breast cancer. The engineered BG@ NbSiR scaffold can eradicate primary tumors, activate the immune response, suppress metastases, prevent tumor relapses (long-term immunological memory) by synergizing with checkpoint blockade immunotherapy, and accelerate osteogenesis as evidenced by multiple in vivo murine models. In particular, single-cell sequencing (scRNA-seq) is employed to further determine the critical factors responding to BG@NbSiR scaffold-based photothermia plus checkpoint blockade-combined immunotherapy.

Several gene functional terms are identified in both tumor biology (including copy number variation) and immune response, which further reveal the underlying therapeutic mechanisms from the perspective of single-cell transcriptome. This work not only demonstrates the promising clinical application potentials of BG@NbSiR scaffold- based therapy against bone metastasis of breast cancer, but also provides distinctive avenues to optimize the design and construction of multifunctional tissue-engineering biomaterials based on single-cell genomes.

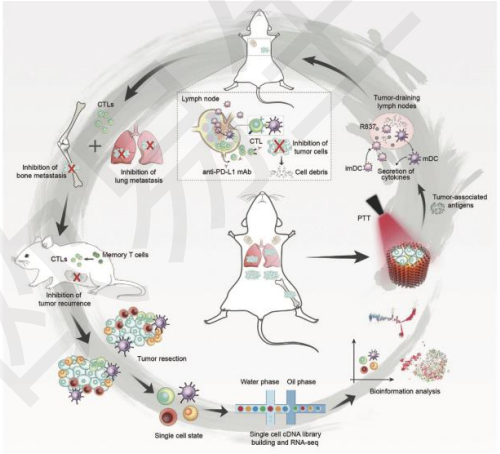

Figure 1. The scheme illustrating the mechanism of anti-tumor immune responses induced by BG@NbSiR-based PTT plus anti-PD-L1 immunotherapy to suppress cancer progression, metastases, and relapse. The fabricated BG@NbSiR scaffolds under the 808 nm NIR laser activation could destroy tumors by thermal effect. Following photonic hyperthermal ablation, large amounts of tumor debris were released and exhibited vaccine-like functions together with R837, which promoted dendritic cells' (DCs) recruitment/maturation and cytokine secretion, activating immune response to attack tumors. Particularly, after integrating with anti-PD-L1 therapy, the anti-tumor immune responses enhanced due to the elevated level of tumor-infiltrating $CD8^+$ cytotoxic T lymphocytes (CTLs). This combined treatment not only inhibited the primary tumors by photothermal therapy (PTT), but also suppressed the tumor metastases in multiple murine models, including distant, lung, and bone metastasis tumors models. In addition, the combined therapy also established long-term protection to avoid tumor relapse through immune memory. Mechanically, scRNA-seq was employed to study the critical factors responding to BG@NbSiR scaffold-based PTT plus checkpoint blockade combined therapy.

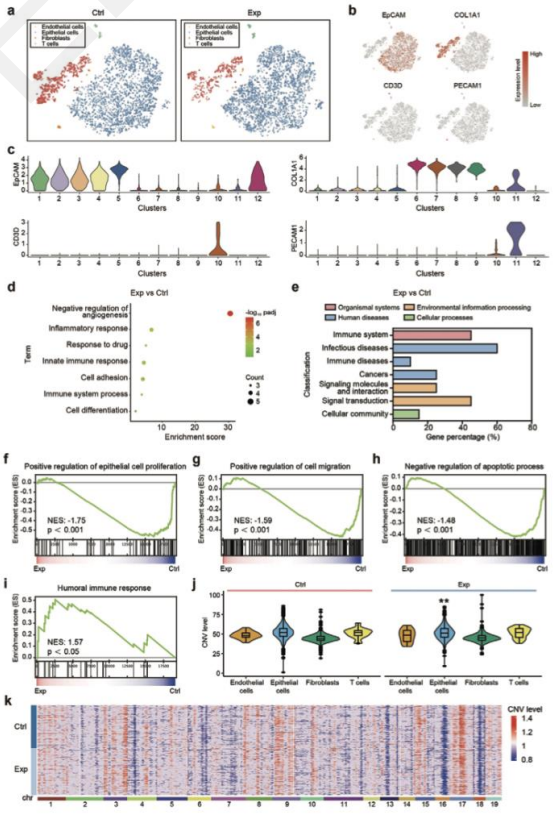

Figure 7. Mechanism of BG@NbSiR-scaffold-based PTT plus anti-PD-L1 immunotherapy depicted by single-cell transcriptomic analysis. a) The *t*-distributed stochastic neighbor embedding (*t*-SNE) plot demonstrating main cell types in the BG (Ctrl) group and the BG@NbSiR + NIR + anti-PD-L1 (Exp) group. b) Heatmap and c) violin plots exhibiting the expression of specific gene markers in each cell type. d) GO, e) KEGG, and f–i) GSEA enrichment analyses based on the differential expression genes between the Exp and the Ctrl groups. j) Violin plots and k) heat map showing distributions of CNV scores among different cell types and chromosomes in the Exp and the Ctrl groups, respectively. $^{**}p < 0.01$ compared with the Ctrl group.

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-18916-5 OPEN

Check for updates

Single-cell RNA sequencing highlights the role of inflammatory cancer-associated fibroblasts in bladder urothelial carcinoma

Zhaohui Chen[1,4], Lijie Zhou[1,2,4], Lilong Liu[1,2], Yaxin Hou[1,2], Ming Xiong[1], Yu Yang[3], Junyi Hu[1,2]✉ & Ke Chen[1,2]✉

Although substantial progress has been made in cancer biology and treatment, clinical outcomes of bladder carcinoma (BC) patients are still not satisfactory. The tumor micro- environment (TME) is a potential target. Here, by single-cell RNA sequencing on 8 BC tumor samples and 3 para tumor samples, we identify 19 different cell types in the BC micro- environment, indicating high intra-tumoral heterogeneity. We find that tumor cells down regulated MHC-II molecules, suggesting that the downregulated immunogenicity of cancer cells may contribute to the formation of an immunosuppressive microenvironment. We also find that monocytes undergo M2 polarization in the tumor region and differentiate. Fur- thermore, the LAMP3 + DC subgroup may be able to recruit regulatory T cells, potentially taking part in the formation of an immunosuppressive TME. Through correlation analysis using public datasets containing over 3000 BC samples, we identify a role for inflammatory cancer-associated fibroblasts (iCAFs) in tumor progression, which is significantly related to poor prognosis. Additionally, we characterize a regulatory network depending on iCAFs. These results could help elucidate the protumor mechanisms of iCAFs. Our results provide deep insight into cancer immunology and provide an essential resource for drug discovery in the future.

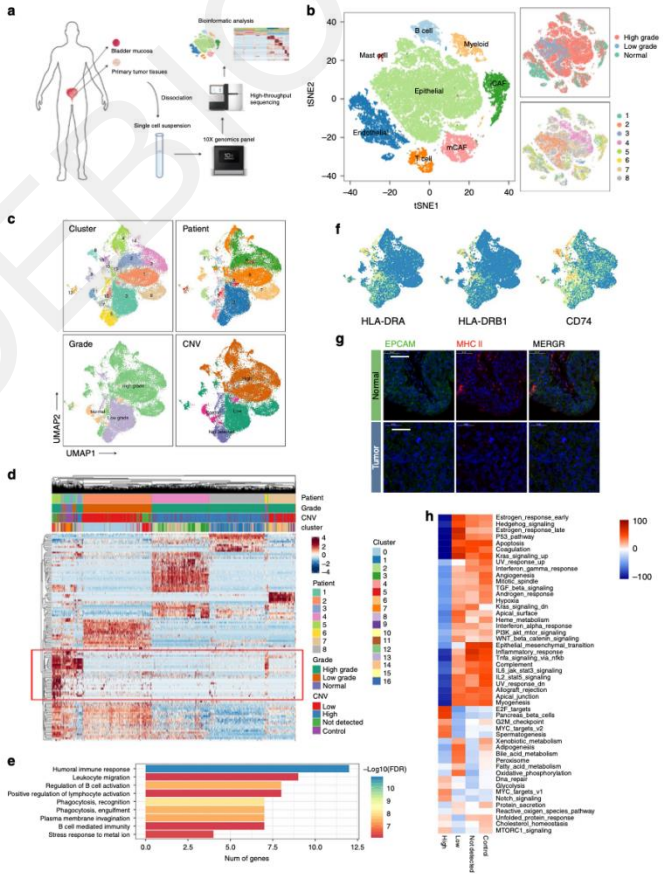

Fig. 1 Identifying infiltrated cell types in BC and non-malignant tissues. a, **b** Identifying infiltrated cell types in BC and non-malignant tissues. **a** Workflow of the sample preparation, sequencing and bioinformatic analysis. **b** tSNE plot of single cells profiled in the presenting work colored by major cell types, tumor grade and patient. **c**–**h** Reclustering of EPCAM+ cells. **c** UMAP plot EPCAM+ cells (epithelial marker) colored by cluster, patient, grade and CNV level. **d** Heatmap of differentially expressed genes (DEGs) of every CNV group. **e** Enriched GO functions of downregulated genes in malignant cells. **f** Expression levels of MHC-II molecules and CD74. **g** Immunofluorescence (IF) staining of MHC-II molecules and EPCAM. Scale bar represents 50 μm. **h** Heatmap shows difference in pathway activities scored by GSVA per cell between different CNV groups. Shown are *t*-values from a lineal model.

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-19414-4 OPEN

Single-cell analysis of developing and azoospermia human testicles reveals central role of Sertoli cells

LiangYu Zhao[1,2,6], ChenCheng Yao[1,2,6], XiaoYu Xing[3,6], Tao Jing[1,4,6], Peng Li[1], ZiJue Zhu[1], Chao Yang[1], Jing Zhai[1], RuHui Tian[1], HuiXing Chen[1], JiaQiang Luo[1], NaChuan Liu[1], ZhiWen Deng[2], XiaoHan Lin[2], Na Li[2], Jing Fang[2,5], Jie Sun[3]✉, ChenChen Wang[5]✉, Zhi Zhou[2]✉ & Zheng Li[1]✉

Clinical efficacy of treatments against non-obstructive azoospermia (NOA), which affects 1% of men, are currently limited by the incomplete understanding of NOA pathogenesis and normal spermatogenic microenvironment. Here, we profile >80,000 human testicular single- cell transcriptomes from 10 healthy donors spanning the range from infant to adult and 7 NOA patients. We show that Sertoli cells, which form the scaffold in the testicular micro- environment, are severely damaged in NOA patients and identify the roadmap of Sertoli cell maturation. Notably, Sertoli cells of patients with congenital causes (Klinefelter syndrome and Y chromosome microdeletions) are mature, but exhibit abnormal immune responses, while the cells in idiopathic NOA (iNOA) are physiologically immature. Furthermore, we find that inhibition of Wnt signaling promotes the maturation of Sertoli cells from iNOA patients, allowing these cells to regain their ability to support germ cell survival. We provide a novel perspective on the development of diagnostic methods and therapeutic targets for NOA.

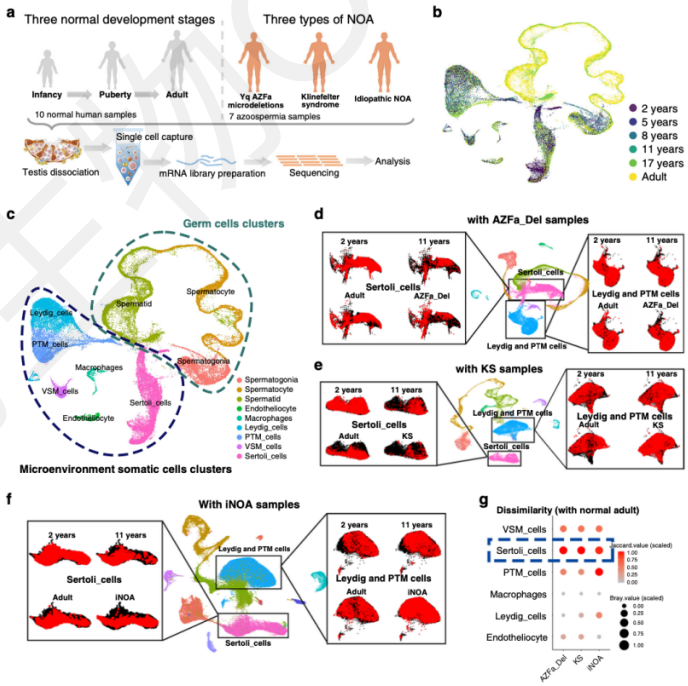

Fig. 1 Global expression profiling of human testicular cells from infancy to adulthood and in NOA patients by single-cell RNA-seq. a Schematic illustration of the experimental workflow. **b**, **c** UMAP plots of all testicular cells from 10 healthy subjects. Cells are colored for **b** ages or **c** types. UMAP, uniform manifold approximation and projection. **d**–**f** UMAP plots of all testicular cells from 10 healthy subjects merged with **d** 1 case of AZFa_Del, **e** 3 cases of KS, or **f** 3 cases of iNOA samples. Sertoli cells (left panels) or Leydig&PTM cells (right panels) are isolated and highlighted as red according to the sample type. **g** Dissimilarity of somatic cells between normal adult and AZFa_Del, KS or iNOA are shown on bubble diagram. The gradient of bubbles sizes indicates low to high scaled Bary value, and the gradient of red indicates low to high scaled Jaccard values. Some elements in panel **a** were downloaded from Servier Medical Art repository. Note: peritubular myoid cells (PTM_cells), vascular smooth muscle cells (VSM_cells); Yq AZFa microdeletions (AZFa_Del); Klinefelter Syndrome (KS); idiopathic NOA (iNOA).

CellPress Partner Journal

Molecular Plant
Research Article

Global Dynamic Molecular Profiling of Stomatal Lineage Cell Development by Single-Cell RNA Sequencing

Zhixin Liu[1,4], Yaping Zhou[1,4], Jinggong Guo[1,4], Jiaoai Li[2,4], Zixia Tian[1], Zhinan Zhu[1], Jiajing Wang[1], Rui Wu[1], Bo Zhang[1], Yongjian Hu[2], Yijing Sun[2], Yan Shangguan[2], Weiqiang Li[1], Tao Li[2], Yunhe Hu[2], Chenxi Guo[1], Jean-David Rochaix[3], Yuchen Miao[1] and Xuwu Sun[1,2,*]

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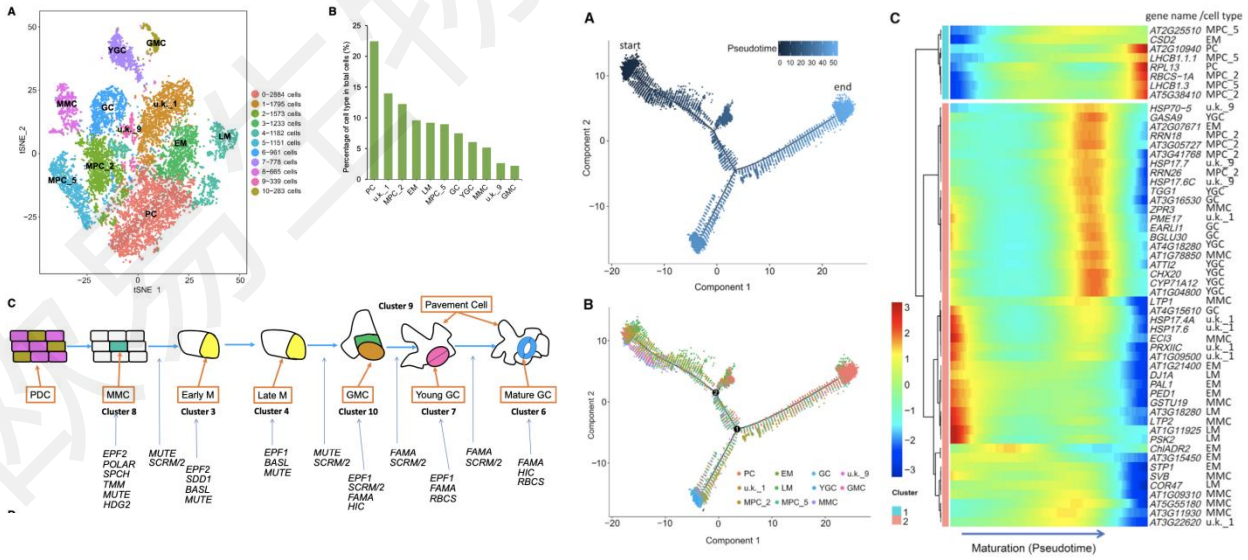
https://doi.org/10.1016/j.molp.2020.06.010

ABSTRACT

The regulation of stomatal lineage cell development has been extensively investigated. However, a comprehensive characterization of this biological process based on single-cell transcriptome analysis has not yet been reported. In this study, we performed RNA sequencing on 12 844 individual cells from the cotyledons of 5-day-old *Arabidopsis* seedlings. We identified 11 cell clusters corresponding mostly to cells at specific stomatal developmental stages using a series of marker genes. Comparative analysis of genes with the highest variable expression among these cell clusters revealed transcriptional networks that regulate development from meristemoid mother cells to guard mother cells. Examination of the developmental dynamics of marker genes via pseudo-time analysis revealed potential interactions between these genes. Collectively, our study opens the door for understanding how the identified novel marker genes participate in the regulation of stomatal lineage cell development.

Keywords: molecular profiling, stomatal, development, single-cell, RNA-seq



Zhu et al. *Cell Death and Disease* (2020)11:829
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Cell Death & Disease

ARTICLE Open Access

Mesenchymal stem cells alleviate LPS-induced acute lung injury by inhibiting the proinflammatory function of Ly6C$^+$ CD8$^+$ T cells

Jiaqi Zhu[1,2], Bing Feng[1,2], Yanping Xu[1,2], Wenyi Chen[1,2], Xinyu Sheng[1,2], Xudong Feng[1,2], Xiaowei Shi[1], Jingqi Liu[1], Qiaoling Pan[1,2], Jiong Yu[1,2], Lanjuan Li[1,2] and Hongcui Cao[1,2,3]

Abstract

Systemic inflammatory processes, including alveolar injury, cytokine induction, and neutrophil accumulation, play key roles in the pathophysiology of acute lung injury (ALI). The immunomodulatory effects of mesenchymal stem cells (MSCs) can contribute to the treatment of inflammatory disorders. In previous studies, the focus was on innate immune cells and the effects of MSCs on ALI through CD8$^+$ T cells remain unclear. In the present study, lipopolysaccharide (LPS) was used to induce ALI in mice. ALI mice were treated with MSCs via intratracheal instillation. Survival rate, histopathological changes, protein levels, total cell count, cytokine levels, and chemokine levels in alveolar lavage fluid were used to determine the efficacy of MSCs. Mass cytometry and single-cell RNA sequencing (scRNA-seq) were used to characterize the CD8$^+$ T cells in the lungs. Ly6C$^-$ CD8$^+$ T cells are prevalent in normal mice, whereas a specialized effector phenotype expressing a high level of Ly6C is predominant in advanced disease. MSCs significantly mitigated ALI and improved survival. MSCs decreased the infiltration of CD8$^+$ T cells, especially Ly6C$^+$ CD8$^+$ T cells into the lungs. Mass cytometry revealed that CD8$^+$ T cells expressing high Ly6C and CXCR3 levels caused tissue damage in the lungs of ALI mice, which was alleviated by MSCs. The scRNA-seq showed that Ly6C$^+$ CD8$^+$ T cells exhibited a more activated phenotype and decreased expression of proinflammatory factors that were enriched the most in immune chemotaxis after treatment with MSCs. We showed that CD8$^+$ T cells play an important role in MSC-mediated ALI remission, and both infiltration quantity and proinflammatory function were inhibited by MSCs, indicating a potential mechanism for therapeutic intervention.

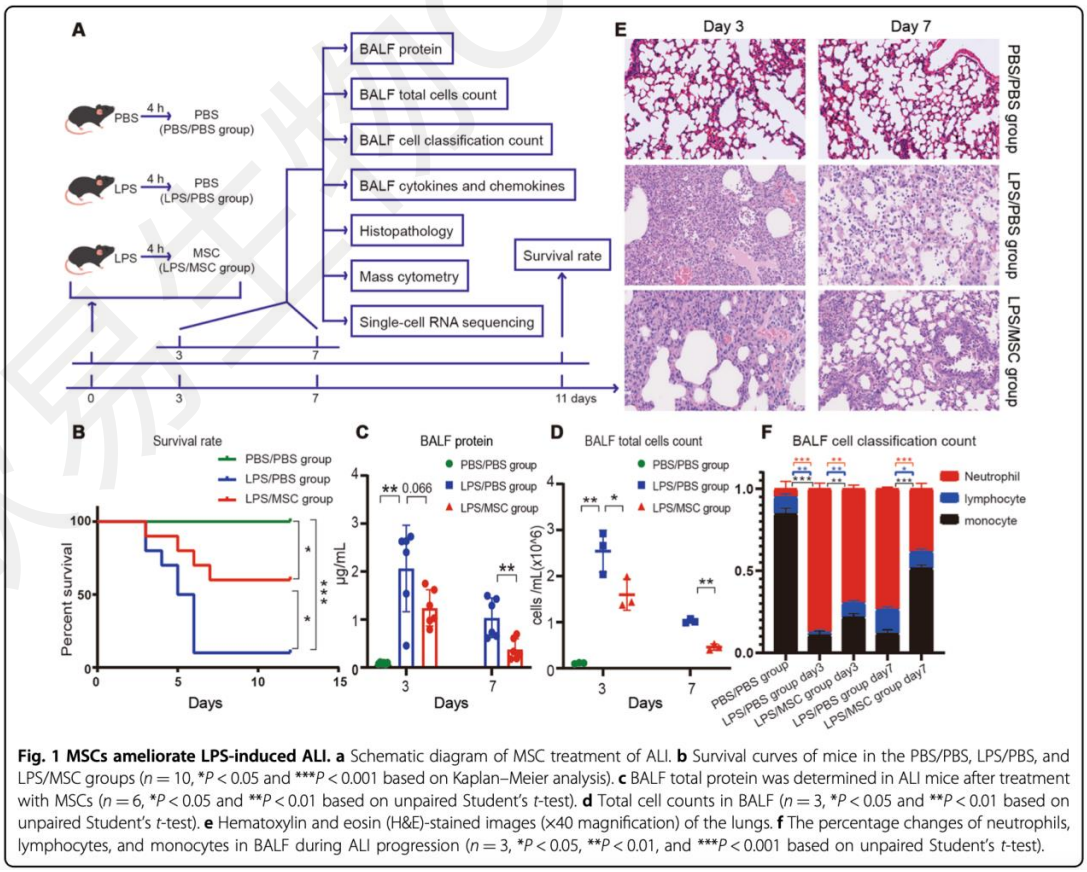

Fig. 1 MSCs ameliorate LPS-induced ALI. a Schematic diagram of MSC treatment of ALI. **b** Survival curves of mice in the PBS/PBS, LPS/PBS, and LPS/MSC groups ($n = 10$, $^*P < 0.05$ and $^{***}P < 0.001$ based on Kaplan–Meier analysis). **c** BALF total protein was determined in ALI mice after treatment with MSCs ($n = 6$, $^*P < 0.05$ and $^{**}P < 0.01$ based on unpaired Student's *t*-test). **d** Total cell counts in BALF ($n = 3$, $^*P < 0.05$ and $^{**}P < 0.01$ based on unpaired Student's *t*-test). **e** Hematoxylin and eosin (H&E)-stained images (×40 magnification) of the lungs. **f** The percentage changes of neutrophils, lymphocytes, and monocytes in BALF during ALI progression ($n = 3$, $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ based on unpaired Student's *t*-test).

Lu et al. *Cell Discovery* (2020)6:69
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Cell Discovery
www.nature.com/celldisc

ARTICLE Open Access

Single-cell transcriptome atlas of lung adenocarcinoma featured with ground glass nodules

Tao Lu[1], Xiaodong Yang[1], Yu Shi[1], Mengnan Zhao[1], Guoshu Bi[1], Jiaqi Liang[1], Zhencong Chen[1], Yiwei Huang[1], Wei Jiang[1], Zongwu Lin[1], Junjie Xi[1], Shuai Wang[1], Yong Yang[2,3], Cheng Zhan[1], Qun Wang[1] and Lijie Tan[1]

Abstract

As an early type of lung adenocarcinoma, ground glass nodule (GGN) has been detected increasingly and now accounts for most lung cancer outpatients. GGN has a satisfactory prognosis and its characteristics are quite different from solid adenocarcinoma (SADC). We compared the GGN adenocarcinoma (GGN-ADC) with SADC using the single-cell RNA sequencing (scRNA-seq) to fully understand GGNs. The tumor samples of five patients with lung GGN-ADCs and five with SADCs underwent surgery were digested to a single-cell suspension and analyzed using 10× Genomic scRNA-seq techniques. We obtained 60,459 cells and then classified them as eight cell types, including cancer cells, endothelial cells, fibroblasts, T cells, B cells, Nature killer cells, mast cells, and myeloid cells. We provided a comprehensive description of the cancer cells and stromal cells. We found that the signaling pathways related to cell proliferation were downregulated in GGN-ADC cancer cells, and stromal cells had different effects in GGN-ADC and SADC based on the analyses of scRNA-seq results. In GGN-ADC, the signaling pathways of angiogenesis were downregulated, fibroblasts expressed low levels of some collagens, and immune cells were more activated. Furthermore, we used flow cytometry to isolate the cancer cells and T cells in 12 GGN-ADC samples and in an equal number of SADC samples, including $CD4^+$ T and $CD8^+$ T cells, and validated the expression of key molecules by quantitative real-time polymerase chain reaction analyses. Through comprehensive analyses of cell phenotypes in GGNs, we provide deep insights into lung carcinogenesis that will be beneficial in lung cancer prevention and therapy.

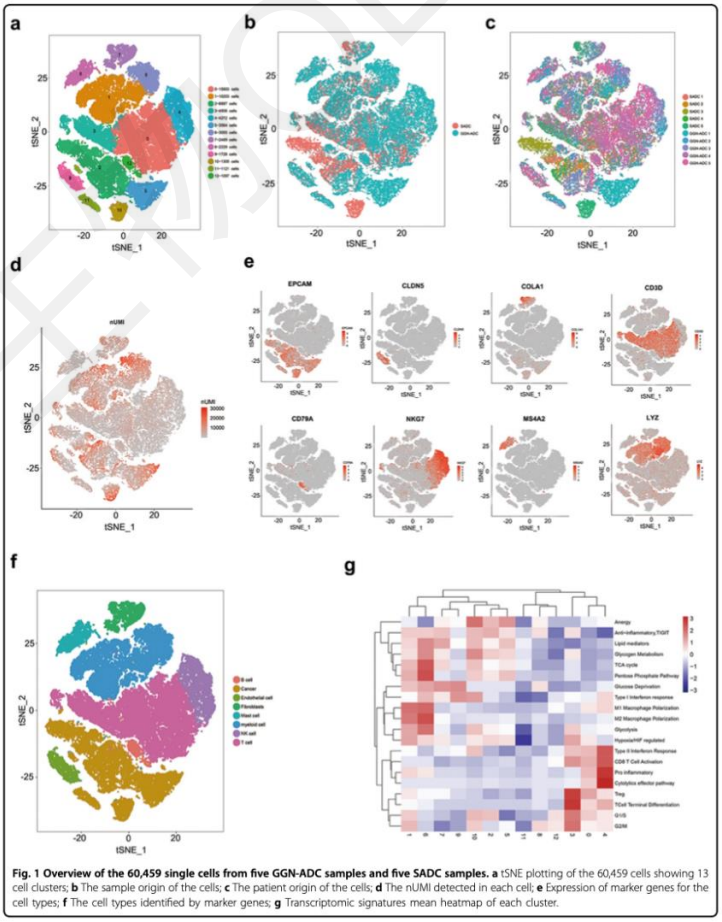

Fig. 1 Overview of the 60,459 single cells from five GGN-ADC samples and five SADC samples. a tSNE plotting of the 60,459 cells showing 13 cell clusters; **b** The sample origin of the cells; **c** The patient origin of the cells; **d** The nUMI detected in each cell; **e** Expression of marker genes for the cell types; **f** The cell types identified by marker genes; **g** Transcriptomic signatures mean heatmap of each cluster.

Feng *et al. Stem Cell Research & Therapy* (2020) 11:418
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Stem Cell Research & Therapy

RESEARCH Open Access

Immunosuppressive effects of mesenchymal stem cells on lung B cell gene expression in LPS-induced acute lung injury

Bing Feng[1,2], Jiaqi Zhu[1,2], Yanping Xu[1,2], Wenyi Chen[1,2], Xinyu Sheng[1,2], Xudong Feng[1,2], Xiaowei Shi[1], Jingqi Liu[1], Qiaoling Pan[1,2], Jinfeng Yang[1,2], Jiong Yu[1,2], Lanjuan Li[1,2] and Hongcui Cao[1,2,3*]

Abstract

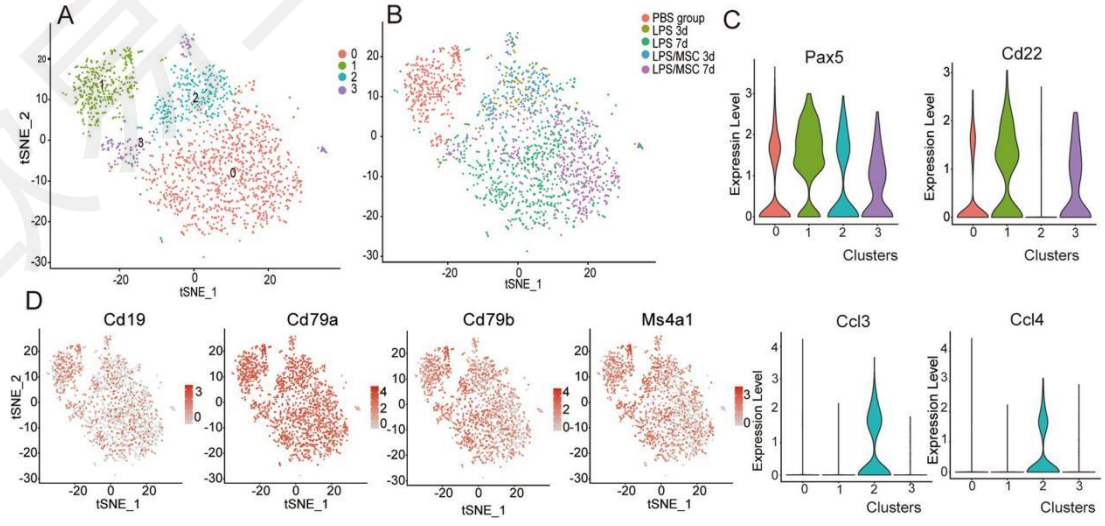
Background: Immune system disorders play important roles in acute lung injury (ALI), and mesenchymal stem cell (MSC) treatment can reduce inflammation during ALI. In this study, we compared the changes in lung B cells during MSC treatment.

Methods: We investigated the effects of MSCs on lung B cells in a mouse model of lipopolysaccharide (LPS)-induced ALI. MSCs were administered intratracheally 4 h after LPS. As vehicle-treated controls, mice were treated with phosphate-buffered saline (PBS) containing 2% C57BL/6 (PBS group). Histopathological changes, survival rate, inflammatory factor levels, and the number of neutrophils in bronchoalveolar lavage fluid (BALF) were determined. Single-cell RNA sequencing (scRNA-Seq) analysis was performed to evaluate the transcriptional changes in lung B cells between the PBS, LPS, and LPS/MSC groups on days 3 and 7.

Results: MSC treatment ameliorated LPS-induced ALI, as indicated by the reductions in mortality, the levels of chemokines and cytokines in BALF, and the severity of lung tissue histopathology in ALI mice. Lung B cells in the PBS group remained undifferentiated and had an inhibitory phenotype. Based on our scRNA-Seq results, the differentially expressed genes (DEGs) in lung B cells in both the PBS group and LPS group were involved in chemotaxis processes and some proinflammatory pathways. MSC treatment inhibited the expression of chemokine genes that were upregulated by LPS and were related to the recruitment of neutrophils into lung tissues. Immunoglobulin-related gene expression was decreased in lung B cells of mice treated with LPS/MSC for 7 days. The DEGs regulated by MSCs were enriched in biological processes, including humoral immune response and apoptotic signaling.

Conclusions: Lung B cells played an important role in the effects of treatment of ALI with MSCs. These observations provide new insights into the mechanisms underlying the effects of MSC treatment for ALI.

Keywords: Mesenchymal stem cells, Lung B cells, Single-cell RNA sequencing, Acute lung injury



frontiers in Immunology

ORIGINAL RESEARCH
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The T Cell Receptor Immune Repertoire Protects the Liver From Reconsitution

Qing Liang[1†], Yudi Hu[1†], Meina Zhang[1], Chunjie Lin[2], Wei Zhang[3], Ying Li[4], Ping Zhu[5], Pengxin Xue[1], Yujie Chen[1], Qiyuan Li[1] and Kejia Wang[1]*

[1] National Institute for Data Science in Health and Medicine, School of Medicine, Xiamen University, Xiamen, China, [2] State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Xiamen, China, [3] Department of Pathology, The 971 Hospital of People's Liberation Army Navy, Qingdao, China, [4] Department of Pathology, Qingdao Municipal Hospital, Qingdao, China, [5] Department of Gynaecology and Obstetrics, The 971 Hospital of People's Liberation Army Navy, Qingdao, China

Aberrant immune cell infiltrates and microcircumstances represent characteristic features of liver fibrosis. In this study, we profiled the transcriptomes of intrahepatic CD45+ immune cells, from mice, using single-cell RNA sequencing (scRNA-seq) technology to understand the landscape of intrahepatic immune cells during the pathogenesis of fibrosis. Analysis of approximately 10,000 single-cell transcriptomes revealed an increase in dendritic cells (DCs), macrophages, and neutrophils and a decrease in T and natural killer T (NKT) cells. In addition, we report changes in the transcriptomes of diverse immune cell types, implying a deteriorating intrahepatic immune microcircumstance. Furthermore, we uncovered a novel fibrosis-associated CD8 T (Ccl5+, Ccl4+) and CD4 T (mt-Co1+) cell subpopulation, which infiltrates fibrotic liver and is characterized by abnormal activation or inactivation as well as a TCR decline.

The results from scRNA-seq and bulk immune repertoire sequencing (IR-seq) revealed an obvious decline in T cell receptor (TCR) clonotypes combined with shrinking VJ and VDJ segment usage, as well as lower complementarity-determining region 3 (CDR3) amino acid (AA) diversity from fibrotic liver. Interestingly, a deficiency of TCR IR (TcrbKO mice) led to a deterioration of liver fibrosis, coupled with activation of hepatic stellate cells (HSCs) induced by the upregulation of macrophage and gd T cell distribution in fibrotic TcrbKO livers. Our findings reveal the landscape and dynamics of single immune cells in liver fibrosis, and clarify the protective role of TCR IR in response to chronic liver injury.

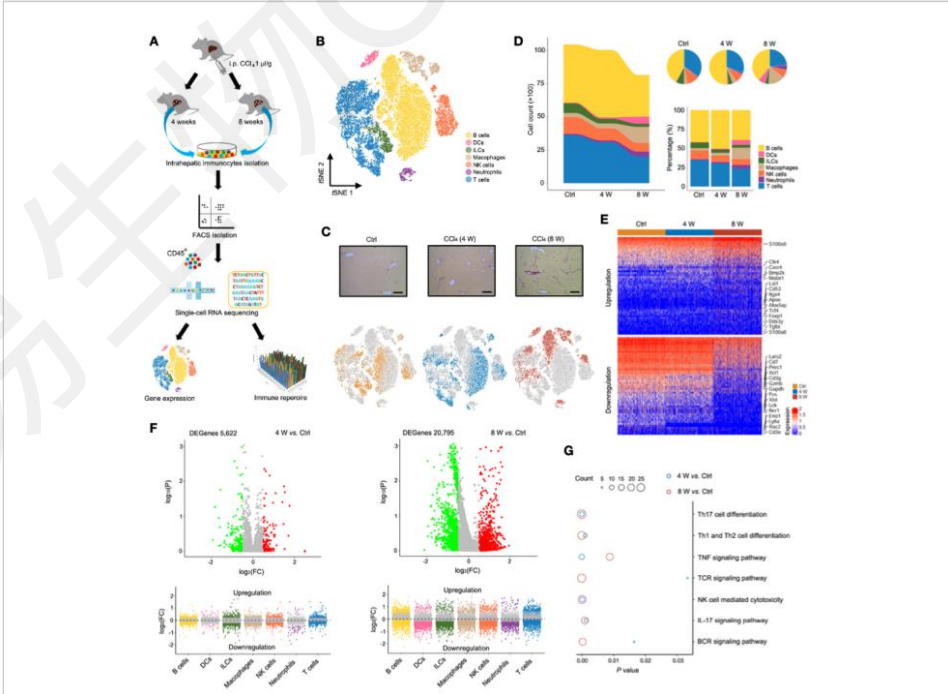

FIGURE 1 | Single-cell atlas of intrahepatic immune cells. **(A)** Flow chart of scRNA-seq and IR-seq. **(B)** scRNA-seq of intrahepatic immune cells from untreated and 4- and 8-week CCl_4-treated livers. *t*SNE clustering of 28,608 single-cell transcriptomes (Ctrl: 10,436 cells, 4-week: 10,028 cells and 8-week: 8,144 cells, n = 4) colored according to cell-type clusters. **(C)** Representative Sirius red staining of untreated, 4- and 8-weeks CCl_4-treated livers (up). *t*SNE clustering as in b, but colored according to groups (down). **(D)** The number and percentage of each cell type. **(E)** Heatmap showing Z-scored mean expression of differentially expressed genes (DEGenes top 50). **(F)** A volcano plot showing DEGenes among the groups (up). Liver fibrosis-dependent gene expression in each cell type (down). Green dots indicate significantly decreased DEGenes, while red dots indicate significantly increased DEGenes. **(G)** Pathway enrichment for the DEGenes among the groups. Circle size indicates enrichment gene number (complete pathway enrichment available in **Table S3**).

frontiers in Plant Science

METHODS
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Single-Cell RNA Sequencing Efficiently Predicts Transcription Factor Targets in Plants

Yunjie Xie[1,2,3,4,5,6,7,8†], Shenfei Jiang[1,2,3,4,5,6,7,8†], Lele Li[2,3,4,5,6,7,8,9†], Xiangzhen Yu[1,2,3,4,5,6,7,8], Yupeng Wang[1,2,3,4,5,6,7,8], Cuiqin Luo[1,2,3,4,5,6,7,8], Qiuhua Cai[2,3,4,5,6,7,8], Wei He[2,3,4,5,6,7,8], Hongguang Xie[2,3,4,5,6,7,8], Yanmei Zheng[1,2,3,4,5,6,7,8], Huaan Xie[1,2,3,4,5,6,7,8,9] and Jianfu Zhang[1,2,3,4,5,6,7,8,9]*

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Discovering transcription factor (TF) targets is necessary for the study of regulatory pathways, but it is hampered in plants by the lack of highly efficient predictive technology. This study is the first to establish a simple system for predicting TF targets in rice (*Oryza sativa*) leaf cells based on 10 × Genomics' single-cell RNA sequencing method. We effectively utilized the transient expression system to create the differential expression of a TF (OsNAC78) in each cell and sequenced all single cell transcriptomes. In total, 35 candidate targets having strong correlations with OsNAC78 expression were captured using expression profiles. Likewise, 78 potential differentially expressed genes were identified between clusters having the lowest and highest expression levels of OsNAC78. A gene overlapping analysis identified 19 genes as final candidate targets, and various assays indicated that Os01g0934800 and Os01g0949900 were OsNAC78 targets. Additionally, the cell profiles showed extremely similar expression trajectories between OsNAC78 and the two targets. The data presented here provide a high-resolution insight into predicting TF targets and offer a new application for single-cell RNA sequencing in plants.

Keywords: transcription factor, TF targets, OsNAC78, expression trajectory, scRNA-seq

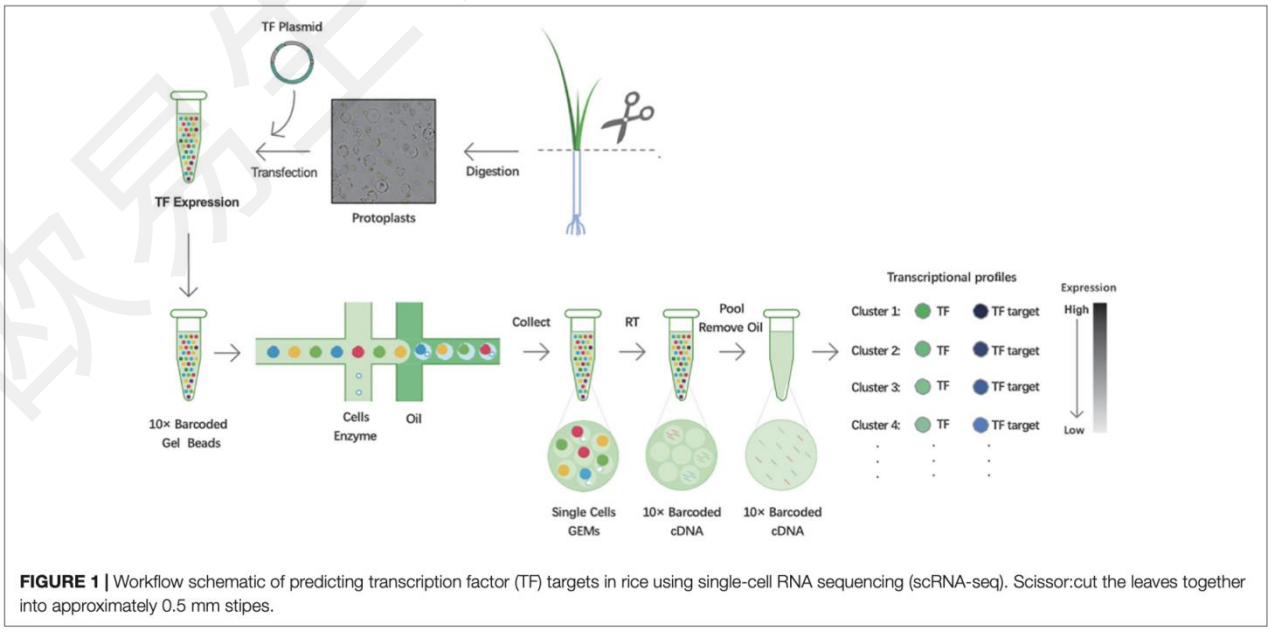


FIGURE 1 | Workflow schematic of predicting transcription factor (TF) targets in rice using single-cell RNA sequencing (scRNA-seq). Scissor:cut the leaves together into approximately 0.5 mm stipes.

Laboratory Investigation
https://doi.org/10.1038/s41374-020-0428-1

ARTICLE

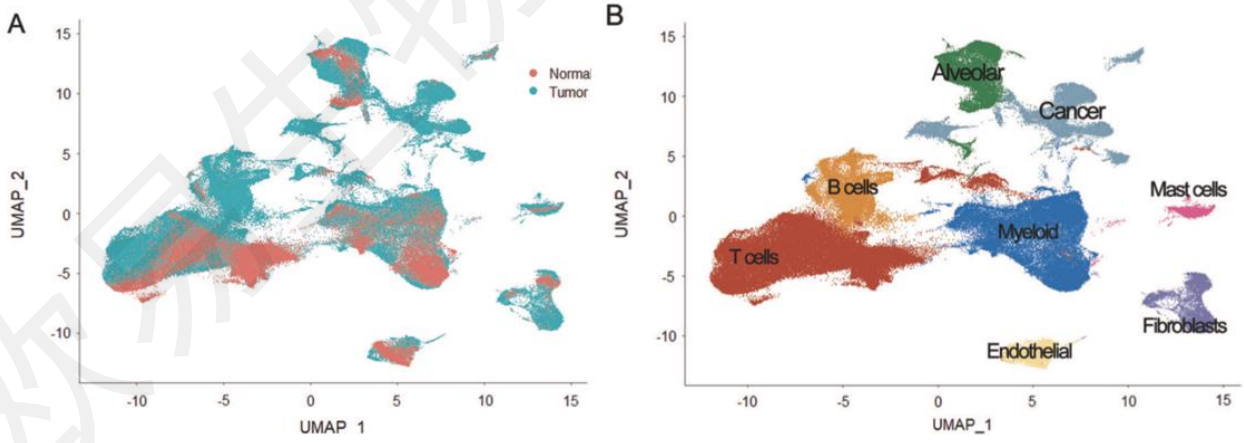
Identification of differentially expressed genes in lung adenocarcinoma cells using single-cell RNA sequencing not detected using traditional RNA sequencing and microarray

Zhencong Chen[1] · Mengnan Zhao[1] · Ming Li[1] · Qihai Sui[1] · Yunyi Bian[1] · Jiaqi Liang[1] · Zhengyang Hu[1] · Yuansheng Zheng[1] · Tao Lu[1] · Yiwei Huang[1] · Cheng Zhan[1] · Wei Jiang[1] · Qun Wang[1] · Lijie Tan[1]

Received: 16 January 2020 / Revised: 28 March 2020 / Accepted: 28 March 2020

Abstract

Lung adenocarcinoma (LUAD) is the leading cause of cancer-related deaths worldwide. Traditional RNA sequencing data fails to detect the exact cellular and molecular changes in tumor cells as they make up only a small proportion of tumor tissue. 10× genomics single-cell RNA sequencing (10× scRNA-seq) and gene expression data of LUAD patients was obtained from the Department of Thoracic Surgery, Zhongshan Hospital, Fudan University, ArrayExpress, TCGA, and GEO databases. Differentially expressed genes (DEGs) were identified in LUAD and alveolar cells (DEGs-scRNA-cancer_cell), tumor- and normal tissue-derived cells (DEGs-scRNA-sample), and normal and LUAD patients (DEGs-Bulk). Flow cytometry and qRT-PCR were performed to validate the significantly differentially expressed ligand–receptor pairs. We selected 159,219 cells and 594 samples in the scRNA-seq data and traditional RNA sequencing, respectively. A total of 1042 DEGs-scRNA-cancer_cell, 788 DEGs-scRNA-sample, and 2510 DEGs-Bulk were identified in this study. We also identified 57 DEGs that were only detected in DEGs-scRNA-cancer_cell (only-DEGs-scRNA-cancer_cell). To explore the relationship between only-DEGs-scRNA-cancer_cell and survival in LUAD, 14 and 22 only-DEGs-scRNA-cancer_cell, which were closely related with survival in TCGA and GEO cohorts were identified. Functional enrichment analyses showed these DEGs-scRNA-cancer_cells were mainly related to cell proliferation and immunoregulation. Our study detected and compared DEGs at different levels and revealed genes that may regulate tumor development. Our results provide a potential new protocol to determine the contribution of DEGs to cancer progression and to help identify potential therapeutic targets.



Overview of the 1,159,219 single cells from eighteen tumor samples and seven normal samples. a, The sample origin of the cells; b, The cell types identified by marker genes.

Int. J. Biol. Sci. 2020, Vol. 16 2205

IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Biological Sciences

2020; 16(12): 2205-2219. doi: 10.7150/ijbs.42080

Research Paper

Ligand-receptor interaction atlas within and between tumor cells and T cells in lung adenocarcinoma

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Abstract

Purpose: Lung adenocarcinoma (LUAD) is the leading cause of cancer-related deaths worldwide. Although tumor cell–T cell interactions are known to play a fundamental role in promoting tumor progression, these interactions have not been explored in LUAD.

Methods: The 10x genomics single-cell RNA sequencing (scRNA-seq) and gene expression data of LUAD patients were obtained from ArrayExpress, TCGA, and GEO databases. scRNA-seq data were analyzed and infiltrating tumor cells, epithelial cells, and T cells were identified in the tumor microenvironment. Differentially expressed ligand-receptor pairs were identified in tumor cells/normal epithelial cells and tumor T cells/non-tumor T cells based on corresponding scRNA-seq and gene expression data, respectively. These important interactions inside/across cancer cells and T cells in LUAD were systematically analyzed. Furthermore, a valid prognostic machine-learning model based on ligand-receptor interactions was built to predict the prognosis of LUAD patients. Flow cytometry and qRT-PCR were performed to validate the significantly differently expressed ligand-receptor pairs.

Results: Overall, 39,692 cells in scRNA-seq data were included in our study after quality filtering. A total of 65 ligand-receptor pairs (17 upregulated and 48 downregulated), including LAMB1-ITGB1, CD70-CD27, and HLA-B-LILRB2, and 96 ligand-receptor pairs (41 upregulated and 55 downregulated), including CCL5-CCR5, SELPLG-ITGB2, and CXCL13-CXCR5, were identified in LUAD cancer cells and T cells, respectively. To explore the crosstalk between cancer cells and T cells, 114 ligand-receptor pairs, including 11 ligand-receptor pair genes that could significantly affect survival outcomes, were identified in our research. A machine-learning model was established to accurately predict the prognosis of LUAD patients and ITGB4, CXCR5, and MET were found to play an important role in prognosis in our model. Flow cytometry and qRT-PCR analyses indicated the reliability of our study.

Conclusion: Our study revealed functionally significant interactions within and between cancer cells and T cells. We believe these observations will improve our understanding of potential mechanisms of tumor microenvironment contributions to cancer progression and help identify potential targets for immunotherapy in the future.

Key words: Lung adenocarcinoma, Single-cell RNA-seq, Cell-to-cell interactions, Machine learning, Survival

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Theranostics

2021; 11(5): 2232-2246. doi: 10.7150/thno.52514

Research Paper

Mesenchymal stem cell-mediated immunomodulation of recruited mononuclear phagocytes during acute lung injury: a high-dimensional analysis study

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Abstract

Rationale: Acute lung injury (ALI)-recruited mononuclear phagocytes play a pivotal role in lung injury and repair. This study investigated the types of recruited mononuclear phagocytes and the immunotherapeutic effects of allograft mesenchymal stem cells (MSCs) in a mouse model of lipopolysaccharide (LPS)-induced ALI.

Methods: C57BL/6 mice were orotracheally instilled with LPS (20 mg/kg). Compact bone-derived MSCs were administered orotracheally 4 h after LPS inhalation. Mononuclear phagocytes recruited in the lung tissues were characterized at different timepoints by high-dimensional analysis including flow cytometry, mass cytometry, and single-cell RNA sequencing.

Results: Eight mononuclear phagocyte subsets recruited to LPS-challenged lungs were precisely identified. On day 3 after LPS administration, both Ly6C^{hi}CD38^{+} and Ly6C^{low}CD38^{+} monocytes were recruited into acutely injured lungs, which was associated with increased secretion of neutrophil chemokines. Ly6C^{hi}CD38^{+} monocytes differentiated into M1 macrophages on day 3, and subsequently differentiated into CD38^{+} monocyte-derived dendritic cells (mo-DCs) on day 7, while Ly6C^{low}CD38^{+} monocytes differentiated into CD11b^{+}CD38^{+} DCs on day 7. When ALI mice were treated with MSCs, the mortality significantly reduced. Notably, MSCs reduced the amount of M1 macrophages and reduced the secretion of neutrophil chemokines on day 3. Furthermore, MSCs reduced the number of CD38^{+} mo-DCs and CD11b^{+}CD38^{+} DCs on day 7, suppressing the antigen presentation process. Recruited mononuclear phagocyte subsets with a high level of CD38 exhibited an activated phenotype and could secrete higher levels of cytokines and chemokines.

Conclusions: This study characterized the dynamic functions and phenotypes of recruited mononuclear phagocytes in ALI mice and MSC-treated ALI mice.

Key words: Acute lung injury, Recruited mononuclear phagocytes, Mass cytometry, Single-cell RNA sequencing, Mesenchymal stem cells

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DOI: 10.1002/ctm2.350

CLINICAL AND TRANSLATIONAL MEDICINE Open Access WILEY

RESEARCH ARTICLE

Landscape and dynamics of single tumor and immune cells in early and advanced-stage lung adenocarcinoma

Zhencong Chen[#] | Yiwei Huang[#] | Zhengyang Hu[#] | Mengnan Zhao | Ming Li | Guoshu Bi | Yuansheng Zheng | Jiaqi Liang | Tao Lu | Wei Jiang | Songtao Xu | Cheng Zhan[*] | Junjie Xi[*] | Qun Wang | Lijie Tan

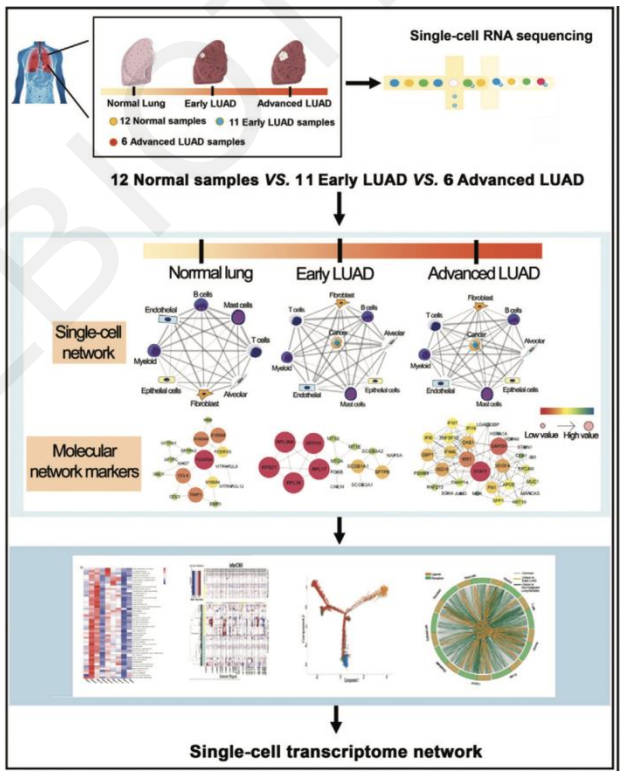
Abstract

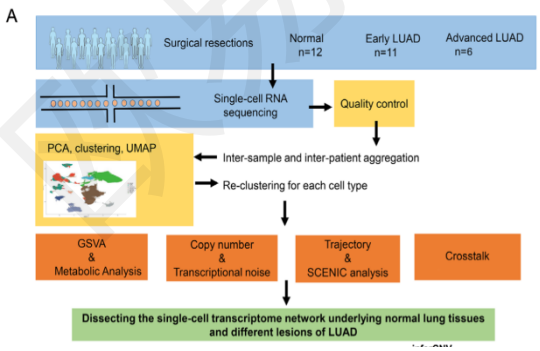
Background: Lung adenocarcinoma (LUAD) patients with different American Joint Committee on Cancer stages have different overall 5-year survival rates. The tumor microenvironment (TME) and intra-tumor heterogeneity (ITH) have been shown to play a crucial role in the occurrence and development of tumors. However, the TME and ITH in different lesions of LUAD have not been extensively explored.

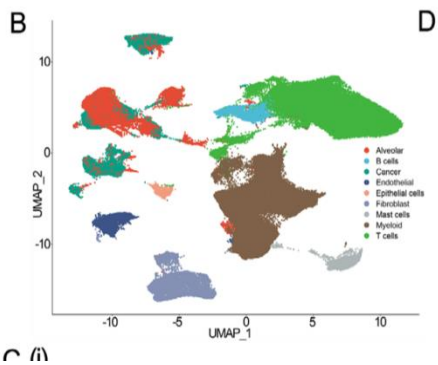
Methods: We present a 204,157-cell catalog of the TME transcriptome in 29 lung samples to systematically explore the TME and ITH in the different stages of LUAD. Traditional RNA sequencing data and complete clinical information were downloaded from publicly available databases.

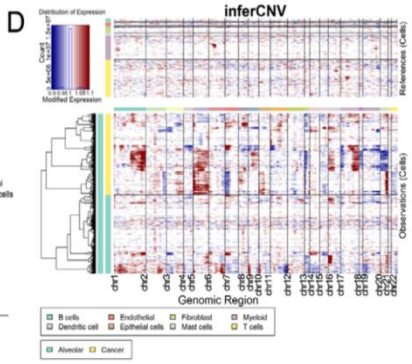
Results: Based on these high-quality cells, we constructed a single-cell network underlying cellular and molecular features of normal lung, early LUAD, and advanced LUAD cells. In contrast with early malignant cells, we noticed that advanced malignant cells had a remarkably more complex TME and higher ITH level. We also found that compared with other immune cells, more differences in CD8+/CTL T cells, regulatory T cells, and follicular B cells were evident between early and advanced LUAD. Additionally, cell-cell communication analyses, revealed great diversity between different lesions of LUAD at the single-cell level. Flow cytometry and qRT-PCR were used to validate our results.

Conclusion: Our results revealed the cellular diversity and molecular complexity of cell lineages in different stages of LUAD. We believe our research, which serves as a basic framework and valuable resource, can facilitate exploration of the pathogenesis of LUAD and identify novel therapeutic targets in the future.









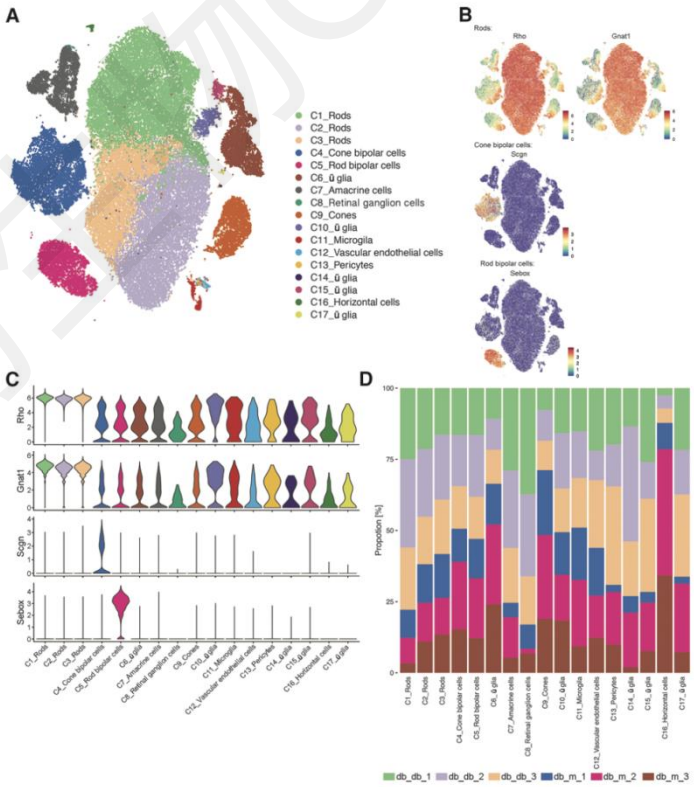
Diabetes Volume 70, May 2021 1185

Pathogenesis Study Based on High-Throughput Single-Cell Sequencing Analysis Reveals Novel Transcriptional Landscape and Heterogeneity of Retinal Cells in Type 2 Diabetic Mice

Tian Niu, Junwei Fang, Xin Shi, Mengya Zhao, Xindan Xing, Yihan Wang, Shaopin Zhu, and Kun Liu

Diabetes 2021;70:1185–1197 | https://doi.org/10.2337/db20-0839

Diabetic retinopathy (DR) is the leading cause of acquired blindness in middle-aged people. The complex pathology of DR is difficult to dissect, given the convoluted cytoarch- itecture of the retina. Here, we performed single-cell RNA sequencing (scRNA-seq) of retina from a model of type 2 diabetes, induced in leptin receptor–deficient (db/db) and control db/m mice, with the aim of elucidating the factors mediating the pathogenesis of DR. We identified 11 cell types and determined cell-type-specific expression of DR-associated loci via genome-wide association study (GWAS)-based enrichment analysis. DR also impacted cell-type-specific genes and altered cell-cell communica- tion. Based on the scRNA-seq results, retinaldehyde-bind- ing protein 1 (RLBP1) was investigated as a promising therapeutic target for DR. Retinal RLBP1 expression was decreased in diabetes, and its overexpression in Mu€ller glia mitigated DR-associated neurovascular degenera- tion. These data provide a detailed analysis of the retina under diabetic and normal conditions, revealing new in- sights into pathogenic factors that may be targeted to treat DR and related dysfunctions.



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DOI: 10.1111/cpr.13007

ORIGINAL ARTICLE

Cell Proliferation WILEY

Single-cell transcriptomes of mouse bladder urothelium uncover novel cell type markers and urothelial differentiation characteristics

Yan Li[1,2] | Yaxiao Liu[1,2,3] | Zhengdong Gao[1,2] | Lekai Zhang[1,2] | Lipeng Chen[1,2] | Zonglong Wu[1,2] | Qinggang Liu[1,2] | Shuai Wang[1,2] | Nan Zhou[1,2] | Toby C. Chai[4] | Benkang Shi[1,2]

Abstract

Objectives: Much of the information to date in terms of subtypes and function of bladder urothelial cells were derived from anatomical location or by the expression of a small number of marker genes. To have a comprehensive map of the cellular anatomy of bladder urothelial cells, we performed single-cell RNA sequencing to thoroughly characterize mouse bladder urothelium.

Materials and methods: A total of 18,917 single cells from mouse bladder urothe- lium were analysed by unbiased single-cell RNA sequencing. The expression of the novel cell marker was confirmed by immunofluorescence using urinary tract infection models.

Results: Unsupervised clustering analysis identified 8 transcriptionally distinct cell subpopulations from mouse bladder urothelial cells. We discovered a novel type of bladder urothelial cells marked by Plxna4 that may be involved with host response and wound healing. We also found a group of basal-like cells labelled by ASPM that could be the progenitor cells of adult bladder urothelium. $ASPM^{+}$ urothelial cells are significantly increased after injury by UPEC. In addition, specific transcription factors were found to be associated with urothelial cell differentiation. At the last, a number of interstitial cystitis/bladder pain syndrome–regulating genes were found differen- tially expressed among different urothelial cell subpopulations.

Conclusions: Our study provides a comprehensive characterization of bladder urothelial cells, which is fundamental to understanding the biology of bladder urothe- lium and associated bladder disease.

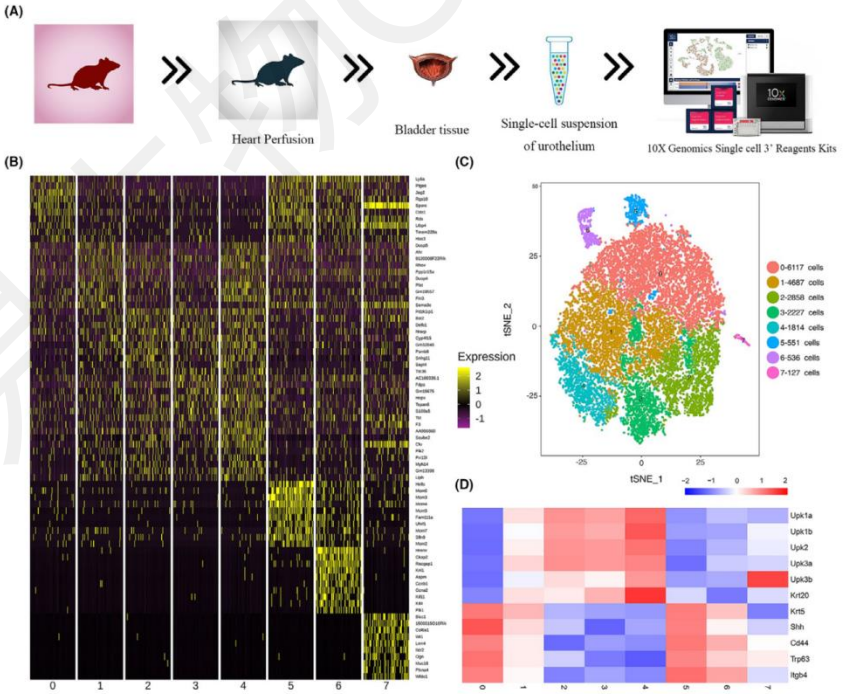

FIGURE 1 Cell diversity of mouse urinary bladder urothelial cells delineated by single-cell transcriptomic analysis (A) schematics of the experimental design for single-cell RNA sequencing. The mouse bladder urothelum were collected and processed into a single-cell suspension. Single-cell RNA sequencing was performed using the 10x-Based Genomics platform. B, Heat map showing the differential genes distinguishing the cell subtypes by using Seurat tool. C, Unsupervised clustering demonstrates 8 subtypes shown in a t-SNE 2D map. (D) Heat map showing the expression of typical urothelial cell type–specific markers genes in each cluster

Liu *et al. Cell Biosci* (2021) 11:51
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Cell & Bioscience

RESEARCH Open Access

Single-cell transcriptomic analysis of eutopic endometrium and ectopic lesions of adenomyosis

Zhiyong Liu[1†], Zhonghua Sun[2†], Hongyun Liu[3], Weipin Niu[1], Xin Wang[4], Na Liang[5], Xin Wang[6], Yanfei Wang[6], Yaxin Shi[6], Li Xu[7*] and Wei Shi[6*]

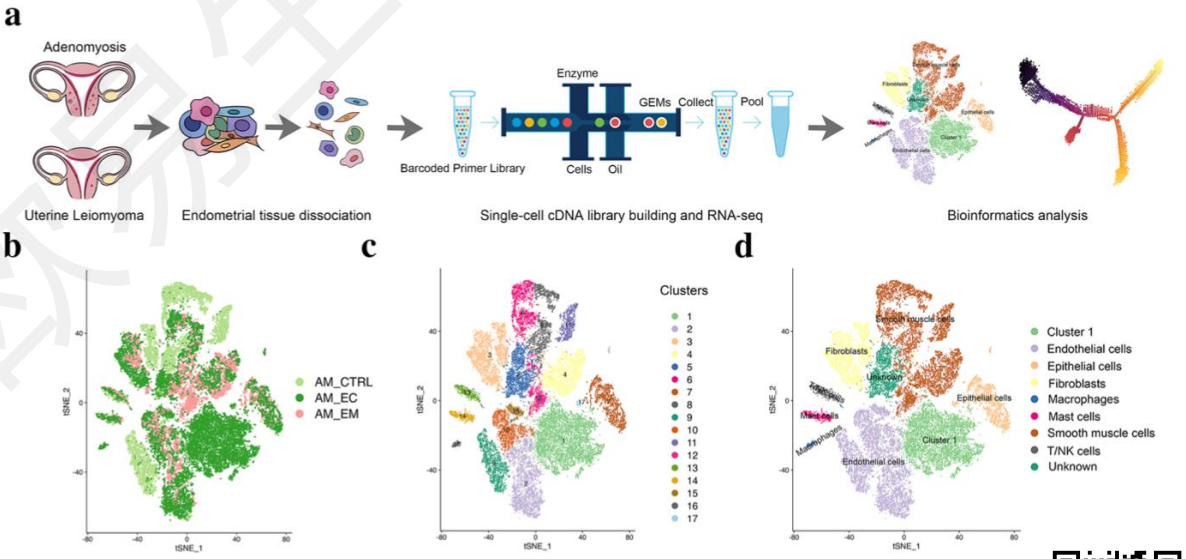
Abstract

Background: Adenomyosis (AM) is a common benign chronic gynaecological disorder; however, the precise pathogenesis of adenomyosis is still poorly understood. Single-cell RNA sequencing (scRNA-seq) can uncover rare subpopulations, explore genetic and functional heterogeneity, and reveal the uniqueness of each cell. It provides us a new approach to reveal biological issues from a more detailed and microscopic perspective. Here, we utilize this revolutionary technology to identify the changes of gene expression patterns between ectopic lesions and the eutopic endometrium at the single-cell level and explore a potential novel pathogenesis of AM.

Methods: A control endometrium (sample with leiomyoma excluding endometrial disorders, n = 1), eutopic endometrium and ectopic lesion (from a patient with adenomyosis, n = 1) samples were analysed by scRNA-seq, and additional leiomyoma (n = 3) and adenomyosis (n = 3) samples were used to confirm colocalization and vasculogenic mimicry (VM) formation. Protein colocalization was visualized by immunofluorescence, and CD34-periodic acid-Schiff (PAS) double staining was used to assess the formation of VM.

Results: The scRNA-seq results suggest that cancer-, cell motility- and inflammation- (CMI) associated terms, cell proliferation and angiogenesis play important roles in the progression of AM. Moreover, the colocalization of EPCAM and PECAM1 increased significantly in the ectopic endometrium group ($P < 0.05$), cell subpopulation with high copy number variation (CNV) levels possessing tumour-like features existed in the ectopic lesion sample, and VNN1- and EPCAM-positive cell subcluster displayed active cell motility in endometrial epithelial cells. Furthermore, during the transformation of epithelial cells to endothelial cells, we observed the significant accumulation of VM formation (positively stained with PAS but not CD34, $P < 0.05$) in ectopic lesions.

Conclusions: In the present study, our results support the theory of adenomyosis derived from the invasion and migration of the endometrium. Moreover, cell subcluster with high CNV level and tumour-associated characteristics is identified. Furthermore, epithelial-endothelial transition (EET) and the formation of VM in tumours, the latter of



www.aging-us.com AGING 2021, Vol. 13, Advance

Research Paper

Phenotyping of immune and endometrial epithelial cells in endometrial carcinomas revealed by single-cell RNA sequencing

Yu-e Guo[2,*], Yiran Li[2,*], Bailian Cai[1], Qizhi He[3], Guofang Chen[1], Mengfei Wang[1], Kai Wang[1], Xiaoping Wan[2], Qin Yan[2,&]

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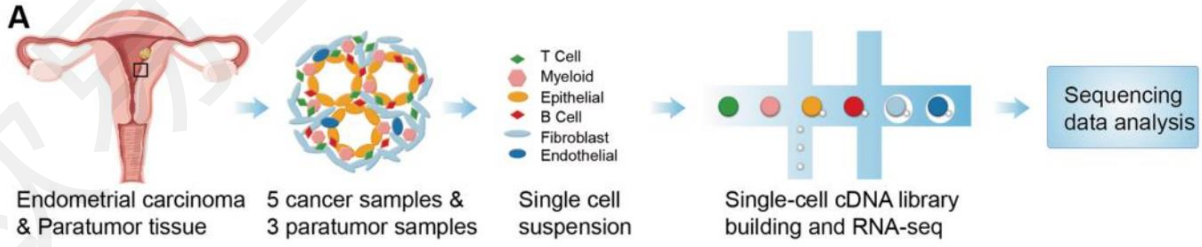
*Equal contribution

ABSTRACT

Tumors are complex ecosystems harboring multiple cell types which might play a critical role in tumor progression and treatment response. The endometrial epithelial cell identities and immune microenvironment of endometrial carcinoma (ECC) are poorly characterized. In this study, a cellular map of endometrial carcinoma was generated by profiling 30,780 cells isolated from tumor and paratumor tissues from five patients using single-cell RNA sequencing. 7 cell types in lymphocytes, 7 types in myeloid cells and 3 types in endometrial epithelial cells were identified. Distinct CD8$^+$ T cell states and different monocyte-macrophage populations were discovered, among which exhausted CD8$^+$ T cells and macrophages were preferentially enriched in tumor. Both CD8$^+$ T cells and macrophages comport with continuous activation model. Gene expression patterns examination and gene ontology enrichment analysis of endometrial epithelial cells revealed 3 subtypes: stem-like cells, secretory glandular cells and ciliated cells. Overall, our study presents a view of endometrial carcinoma at single-cell resolution that reveals the characteristics of endometrial epithelial cells in the endometrium, and provides a cellular landscape of the tumor immune microenvironment.



RESEARCH ARTICLE Open Access

Blinatumomab-induced T cell activation at single cell transcriptome resolution

Yi Huo[1,2†], Zhen Sheng[1,2†], Daniel R. Lu[3], Daniel C. Ellwanger[3], Chi-Ming Li[3], Oliver Homann[3], Songli Wang[3], Hong Yin[2*] and Ruibao Ren[1*]

Abstract

Background: Bi-specific T-cell engager (BiTE) antibody is a class of bispecific antibodies designed for cancer immunotherapy. Blinatumomab is the first approved BiTE to treat acute B cell lymphoblastic leukemia (B-ALL). It brings killer T and target B cells into close proximity, activating patient's autologous T cells to kill malignant B cells via mechanisms such as cytolytic immune synapse formation and inflammatory cytokine production. However, the activated T-cell subtypes and the target cell-dependent T cell responses induced by blinatumomab, as well as the mechanisms of resistance to blinatumomab therapy are largely unknown.

Results: In this study, we performed single-cell sequencing analysis to identify transcriptional changes in T cells following blinatumomab-induced T cell activation using single cells from both, a human cell line model and a patient-derived model of blinatumomab-mediated cytotoxicity. In total, the transcriptome of 17,920 single T cells from the cell line model and 2271 single T cells from patient samples were analyzed. We found that CD8+ effector memory T cells, CD4+ central memory T cells, naïve T cells, and regulatory T cells were activated after blinatumomab treatment. Here, blinatumomab-induced transcriptional changes reflected the functional immune activity of the blinatumomab-activated T cells, including the upregulation of pathways such as the immune system, glycolysis, IFNA signaling, gap junctions, and IFNG signaling. Co-stimulatory (TNFRSF4 and TNFRSF18) and co-inhibitory (LAG3) receptors were similarly upregulated in blinatumomab-activated T cells, indicating ligand-dependent T cell functions. Particularly, B-ALL cell expression of TNFSF4, which encodes the ligand of T cell co-stimulatory receptor TNFRSF4, was found positively correlated with the response to blinatumomab treatment. Furthermore, recombinant human TNFSF4 protein enhanced the cytotoxic activity of blinatumomab against B-ALL cells.

Conclusion: These results reveal a target cell-dependent mechanism of T-cell activation by blinatumomab and suggest that TNFSF4 may be responsible for the resistant mechanism and a potential target for combination therapy with blinatumomab, to treat B-ALL or other B-cell malignancies.

Keywords: Bi-specific T-cell engager antibody, Acute B cell lymphoblastic leukemia, Blinatumomab, T cell activation, Single-cell RNA-Seq, TNFRSF4

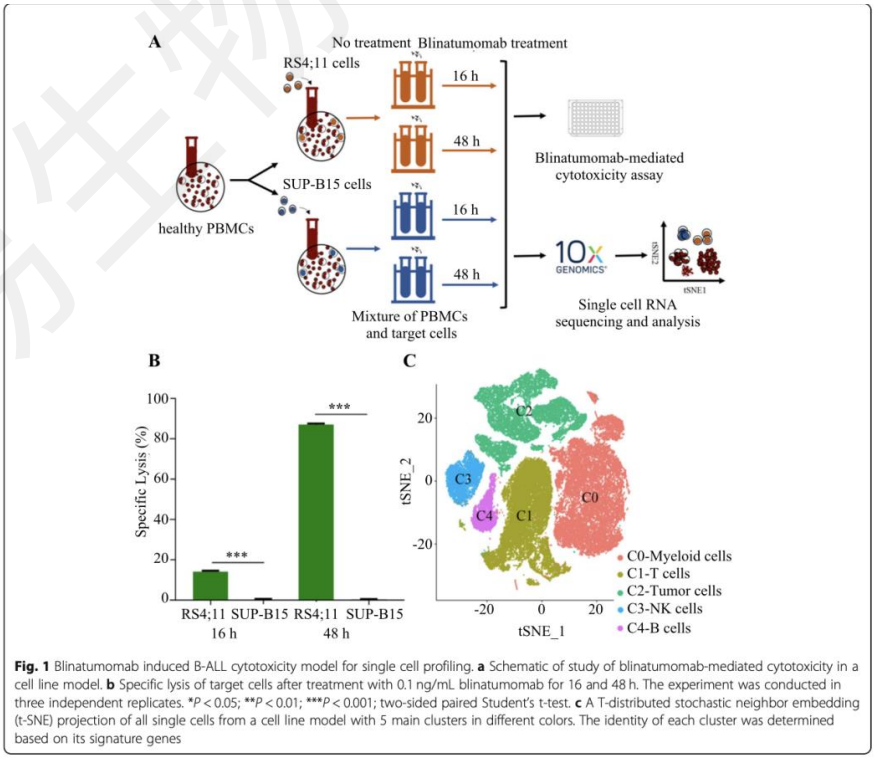


Fig. 1 Blinatumomab induced B-ALL cytotoxicity model for single cell profiling. **a** Schematic of study of blinatumomab-mediated cytotoxicity in a cell line model. **b** Specific lysis of target cells after treatment with 0.1 ng/mL blinatumomab for 16 and 48 h. The experiment was conducted in three independent replicates. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; two-sided paired Student's t-test. **c** A T-distributed stochastic neighbor embedding (t-SNE) projection of all single cells from a cell line model with 5 main clusters in different colors. The identity of each cluster was determined based on its signature genes

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ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

scRNA-seq of ovarian follicle granulosa cells from different fertility goats reveals distinct expression patterns

Zengkuan Li[1] | Junjie Wang[2] | Yong Zhao[2] | Dongxue Ma[1] | Minghui Zhao[2] | Na Li[2] | Yuhao Men[1] | Yuan Zhang[3] | Huimin Chu[4] | Chuzhao Lei[5] | Wei Shen[2] | Othman El-Mahdy Othman[6] | Lingjiang Min[1]

Abstract

The new technology of high-throughput single-cell RNA sequencing (10 × scRNA-seq) was developed recently with many advantages. However, it was not commonly used in farm animal research. There are few reports for the gene expression of goat ovarian follicle granulosa cells (GCs) during different developmental stages. In the current investigation, the gene expression of follicle GCs at different stages from two populations of Ji'ning grey goats: high litter size (HL; ≥3/L; 2 L) and low litter size (LL; ≤2 /L; 2 L) were analysed by scRNA-seq. Many GC marker genes were identified, and the pseudotime showed that GCs developed during the time course which reflected the follicular development and differentiation trajectory. Moreover, the gene expression difference between the two populations HL versus LL was very clear at different developmental stages. Many marker genes differentially expressed at different developmental stages. ASIP and ASPN were found to be highly expressed in the early stage of GCs, INHA, INHBA, MFGE8 and HSD17B1 were identified to be highly expressed in the growing stage of GCs, while IGFBP2, IGFBP5 and CYP11A1 were found to be highly expressed in late stage. These marker genes could be used as reference genes of goat follicle GC development. This investigation for the first time discovered the gene expression patterns in goat follicle GCs in high- or low-fertility populations (based on litter size) by scRNA-seq which may be useful for uncovering the oocyte development potential.

KEYWORDS

goat, granulosa cells, litter size, scRNA-seq

Study Protocol Clinical Trial

Medicine®

OPEN

Study on the clinical mechanism of Tong-Xie-An-Chang Decoction in the treatment of diarrheal irritable bowel syndrome based on single-cell sequencing technology

Xiang Tan, MD[a,b], Xing-jie Zhao, MD[b], Jun-xiang Li, PhD[b], Chun-e Xie, MD[b], Wen-jing Pei, MD[b], Lei Shi, PhD[c], Fu-shun Kou, MD[a,b], Ya-li Yuan, BD[a,b], Xiao-xuan Xue, MD[b,*]

Abstract

Background: Diarrhea-predominant irritable bowel syndrome (IBS-D) is a kind of functional gastrointestinal disorder with obscure pathogenesis, and exploration about differential gene expression and cell heterogeneity of T lymphocytes in peripheral blood in IBS-D patients still remains unknown. Clinicians tend to use symptomatic treatment, but the efficacy is unstable and symptoms are prone to relapse. Traditional Chinese Medicine (TCM) is used frequently in IBS-D with stable and lower adverse effects. Tong-Xie-An-Chang Decoction (TXACD) has been proven to be effective in the treatment of IBS-D. However, the underlying therapeutic mechanism remains unclear. This trial aims to evaluate the clinical efficacy and safety of TXACD in IBS-D and elucidate the gene-level mechanism of IBS-D and therapeutic targets of TXACD based on single-cell sequencing technology.

Methods/design: This is a randomized controlled, double-blind, double-simulation clinical trial in which 72 eligible participants with IBS-D and TCM syndrome of liver depression and spleen deficiency will be randomly allocated in the ratio of 1:1 to two groups: the experimental group and the control group. The experimental group receives Tong-Xie-An-Chang Decoction (TXACD) and Pinaverium bromide tablets placebo; the control group receives pinaverium bromide tablets and TXACD placebo. Each group will be treated for 4 weeks. The primary outcome: the rate of IBS-Symptom Severity Score (IBS-SSS). The secondary outcomes: TCM syndrome score, adequate relief and IBS-Quality of Life Questionnaire (IBS-QOL). Mechanistic outcome is the single-cell sequencing profiling of the T lymphocytes in peripheral blood from IBS-D participants before and after the treatment and healthy individuals.

Discussion: This trial will prove the efficacy and safety of TXACD with high-quality evidence and provide a comprehensive perspective on the molecular mechanism of IBS-D by single-cell sequencing profiling, which makes us pinpoint specific biomarkers of IBS-D and therapeutic targets of TXACD.

Abbreviations: AR = adequate relief, IBS = Irritable bowel syndrome, IBS-D = diarrhea-predominant irritable bowel syndrome, IBS-QOL = IBS-quality of life questionnaire, IBS-SSS = IBS-symptom severity score, PBT = pinaverium bromide tablets, TCM = traditional Chinese Medicine, TXACD = Tong-Xie-An-Chang Decoction.

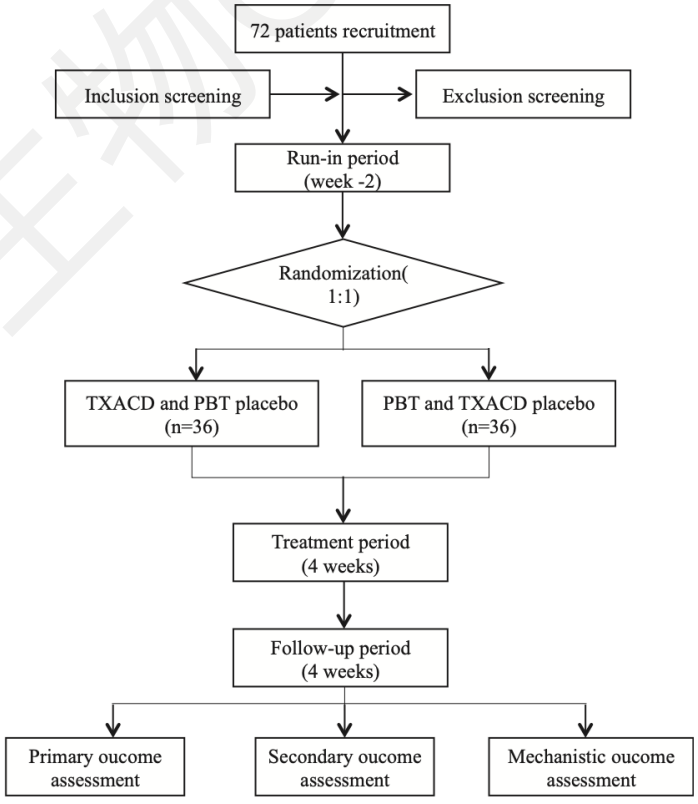

Figure 1. Flowchart of the trial. PBT = pinaverium bromide tablets, TXACD = Tong-Xie-An-Chang Decoction.

frontiers in Endocrinology

ORIGINAL RESEARCH
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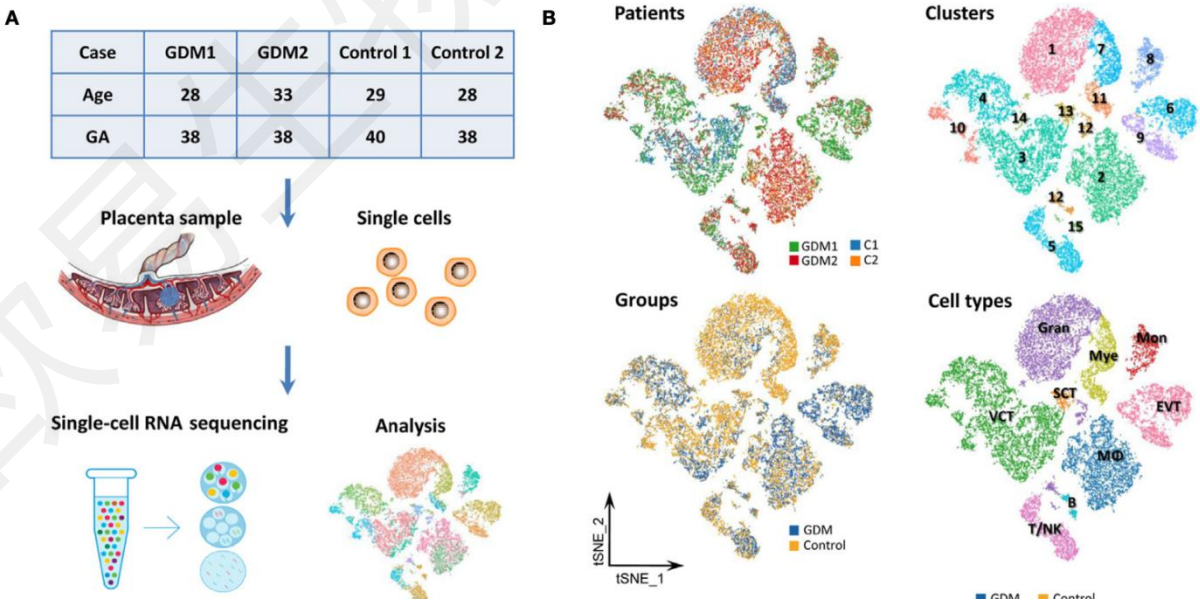
Transcriptomic Profiling of Human Placenta in Gestational Diabetes Mellitus at the Single-Cell Level

OPEN ACCESS

Yuqi Yang[1†‡], Fang Guo[1†‡], Yue Peng[1‡], Rong Chen[2], Wenbo Zhou[1†], Huihui Wang[1], Jun OuYang[1], Bin Yu[1†] and Zhengfeng Xu[3*]*

Gestational diabetes mellitus (GDM) is associated with an increased risk of adverse pregnancy outcomes. Increasing evidence shows that placentation defects may play important roles in GDM. However, our understanding of the human placenta remains limited. In this study, we generated a comprehensive transcriptomic profile of cellular signatures and transcriptomes in the human placenta in GDM using single-cell RNA sequencing (scRNA-seq), constructed a comprehensive cell atlas, and identified cell subtypes and subtype-specific marker genes. In addition, we investigated the placental cellular function and intercellular interactions in GDM. These findings help to elucidate the molecular mechanisms of GDM, and may facilitate the development of new approaches to GDM treatment and prevention.

Keywords: gestational diabetes mellitus, placenta, single-cell RNA sequencing, cellular signatures, transcriptomes



ELSEVIER

The Ocular Surface
Volume 21, July 2021, Pages 206-220

Molecular identity of human limbal heterogeneity involved in corneal homeostasis and privilege

Shengqian Dou [a, b], Qun Wang [a, b], Xia Qi [a, b], Bin Zhang [a, b], Hui Jiang [a, b], Shengwen Chen [a, b], Haoyun Duan [a, b], Yao Lu [c], Jiaoyang Dong [c], Yihai Cao [d], Lixin Xie [a, b], Qingjun Zhou [a, b] ✉, Weiyun Shi [a, b, e] ✉

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Abstract

Purpose

The corneal limbus maintains the homeostasis, immune and angiogenic privilege of cornea. This study aimed to depict the landscape of human limbal tissues by single-cell RNA sequencing (scRNA-seq).

Methods

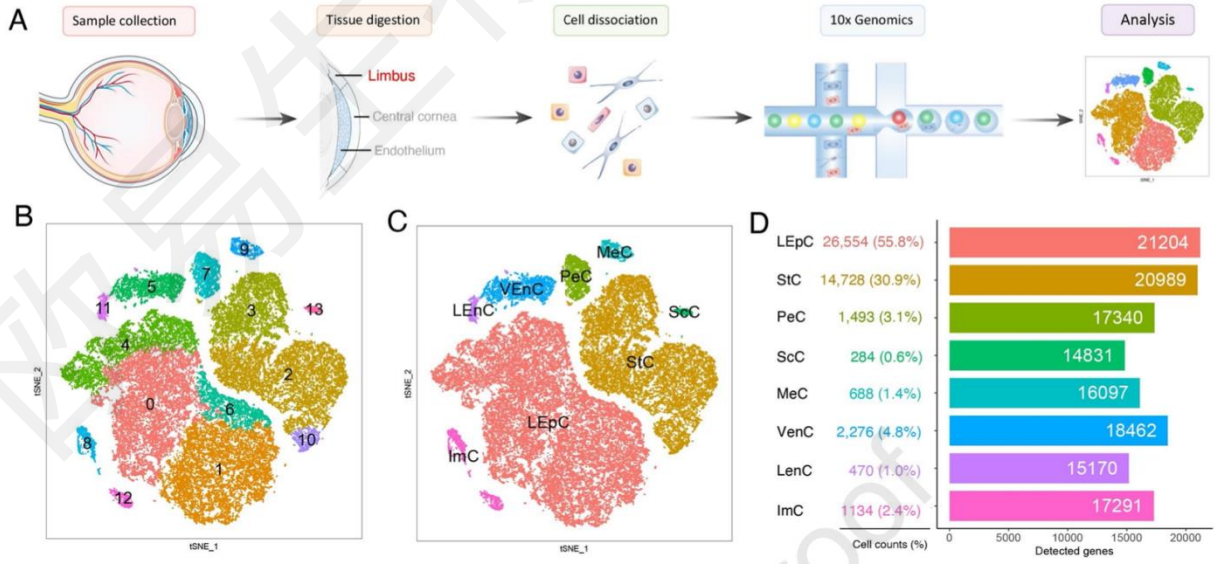
Single cells of human limbus collected from donor corneas were subjected to 10x scRNA-seq, followed by clustering cell types through the t-distributed stochastic neighbor embedding (t-SNE) and unbiased computational informatic analysis. Immunofluorescent staining was performed using human corneas to validate the analysis results.

Results

47,627 cells acquired from six human limbal tissues were collected and subjected to scRNA-seq. 14 distinct clusters were identified and 8 cell types were annotated with representative markers. In-depth dissection revealed three limbal epithelial cell subtypes and refined the X-Y-Z hypothesis of corneal epithelial maintenance. We further unveiled two cell states with higher stemness (*TP63*$^+$ and *CCL20*$^+$ cells), and two other differentiated cell states (*GPHA2*$^+$ and *KRT6B*$^+$ cells) in homeostatic limbal stem/progenitor cells (LSPCs) that differ in transcriptional profiles. Cell-cell communication analysis revealed the central role of LSPCs and their bidirectional regulation with various niche cells. Moreover, comparative analysis between limbus and skin deciphered the pivotal contribution of limbal immune cells, vascular and lymphatic endothelial cells to corneal immune and angiogenic privilege.

Conclusions

The human limbus atlas provided valuable resources and foundations for understanding corneal biology, disease and potential interventions.



ESC European Society of Cardiology
Cardiovascular Research (2022) **118**, 1303–1320
doi:10.1093/cvr/cvab193

Single-cell transcriptomic analyses of cardiac immune cells reveal that Rel-driven CD72-positive macrophages induce cardiomyocyte injury

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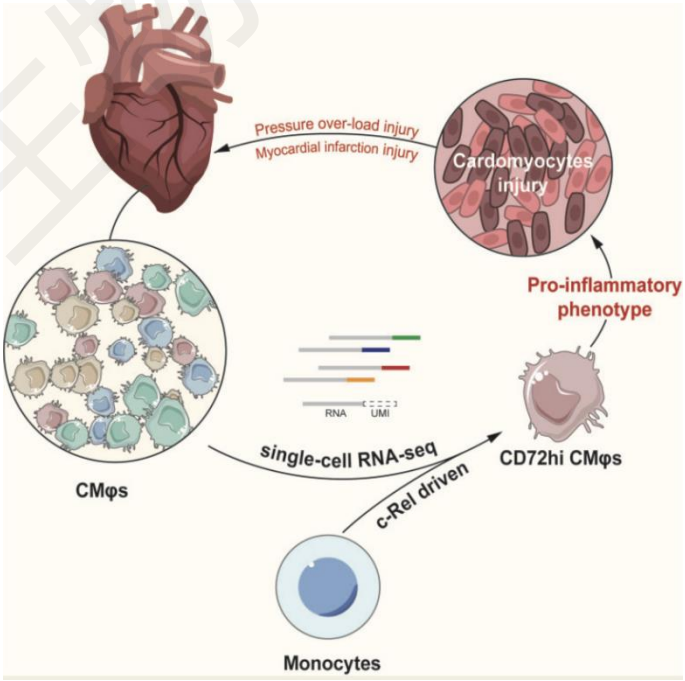
Aims The goal of our study was to investigate the heterogeneity of cardiac macrophages (CMφs) in mice with transverse aortic constriction (TAC) via single-cell sequencing and identify a subset of macrophages associated with heart injury.

Methods and results We selected all CMφs from CD45+ cells using single-cell mRNA sequencing data. Through dimension reduction, clustering, and enrichment analyses, CD72^{hi} CMφs were identified as a subset of pro-inflammatory macrophages. The pseudo-time trajectory and ChIP-Seq analyses identified Rel as the key transcription factor that induces CD72^{hi} CMφ differentiation. Rel KD and Rel-/- bone marrow chimaera mice subjected to TAC showed features of mitigated cardiac injury, including decreased levels of cytokines and ROS, which prohibited cardiomyocyte death. The transfer of adoptive Rel-overexpressing monocytes and CD72^{hi} CMφ injection directly aggravated heart injury in the TAC model. The CD72^{hi} macrophages also exerted pro-inflammatory and cardiac injury effects associated with myocardial infarction. In humans, patients with heart failure exhibit increased CD72^{hi} CMφ levels following dilated cardiomyopathy and ischaemic cardiomyopathy.

Conclusion Bone marrow-derived, Rel-mediated CD72^{hi} macrophages play a pro-inflammatory role, induce cardiac injury and, thus, may serve as a therapeutic target for multiple cardiovascular diseases.



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Research paper

Dissecting the single-cell transcriptome network underlying esophagus non-malignant tissues and esophageal squamous cell carcinoma

Zhencong Chen [a,1], Mengnan Zhao [a,1], Jiaqi Liang [a,1], Zhengyang Hu [a,1], Yiwei Huang [a], Ming Li [a], Yanrui Pang [b], Tao Lu [a], Qihai Sui [a], Cheng Zhan [a,*], Miao Lin [a,*], Weigang Guo [a,*], Qun Wang [a], Lijie Tan [a]

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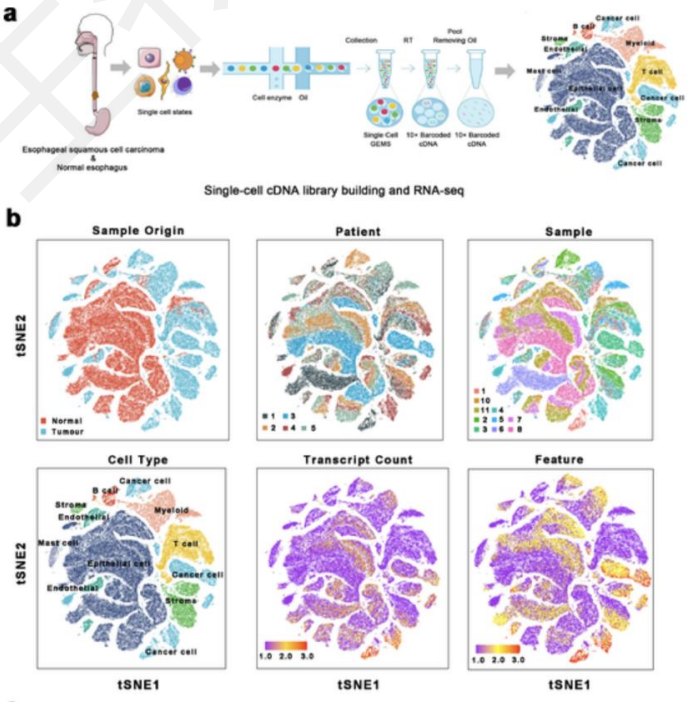
ABSTRACT

Background: Esophageal squamous cell carcinoma (ESCC) is among the most prevalent causes of cancer-related death in adults. Tumor microenvironment (TME) has been associated with therapeutic failure and lethal outcomes for patients. However, published reports on the heterogeneity and TME in ESCC are scanty.
Methods: Five tumor samples and five corresponding non-malignant samples were subjected to scRNA-seq analysis. Bulk RNA sequencing data were retrieved in publicly available databases.
Findings: From the scRNA-seq data, a total of 128,688 cells were enrolled for subsequent analyses. Gene expression and CNV status exhibited high heterogeneity of tumor cells. We further identified a list of tumor-specific genes and four malignant signatures, which are potential new markers for ESCC. Metabolic analysis revealed that energy supply-related pathways are pivotal in cancer metabolic reprogramming. Moreover, significant differences were found in stromal and immune cells between the esophagus normal and tumor tissues, which promoted carcinogenesis at both cellular and molecular levels in ESCC. Immune checkpoints, regarded as potential targets for immunotherapy in ESCC were significantly highly expressed in ESCC, including LAG3 and HAVCR2. Eventually, we constructed a cell-to-cell communication atlas based on cancer cells and immune cells and performed the flow cytometry, qRT-PCR, immunofluorescence, and immunohistochemistry analyses to validate the results.
Interpretation: This study demonstrates a widespread reprogramming across multiple cellular elements within the TME in ESCC, particularly in transcriptional states, cellular functions, and cell-to-cell interactions. The findings offer an insight into the exploration of TME and heterogeneity in the ESCC and provide new therapeutic targets for its clinical management in the future.



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https://doi.org/10.1186/s13578-021-00637-x

Cell & Bioscience

RESEARCH Open Access

Single-cell transcriptomic analysis of endometriosis provides insights into fibroblast fates and immune cell heterogeneity

Junyan Ma[1†], Liqi Zhang[2†], Hong Zhan[2], Yun Mo[2], Zuanjie Ren[2], Anwen Shao[3] and Jun Lin[1*]

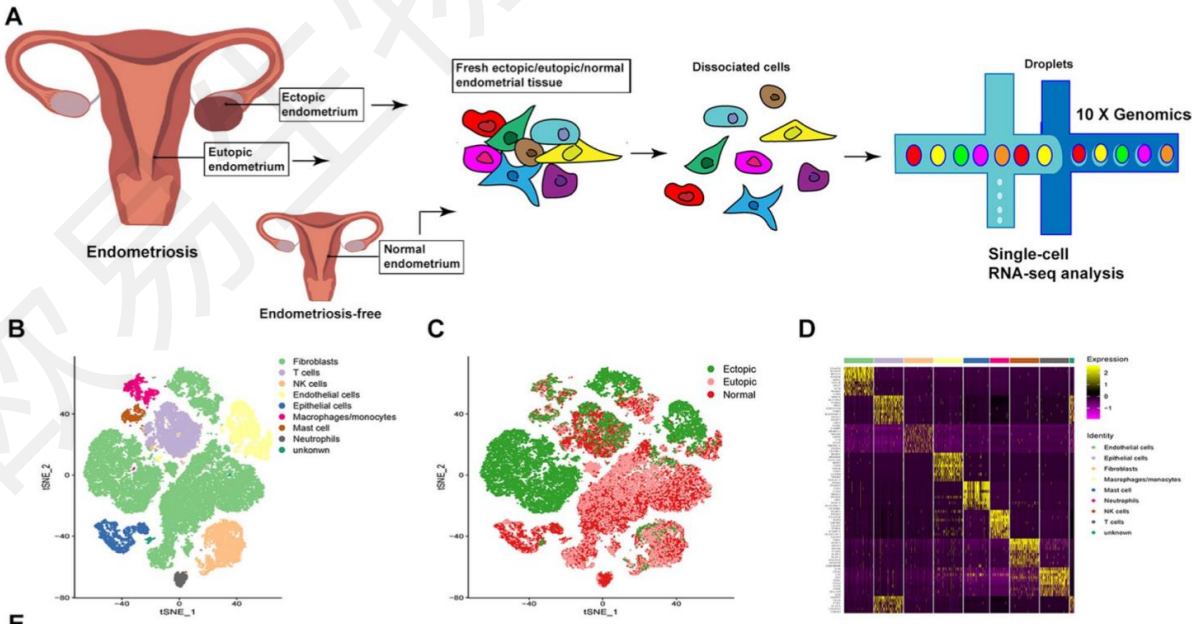
Abstract

Background: Endometriosis is an oestrogen-dependent disease with an unclear aetiology and pathogenesis affecting 6–10% of the global female population, predominantly those of reproductive age. Herein, we profile the transcriptomes of approximately 55,000 single cells from three groups including ectopic endometrium, eutopic endometrium from women with endometriosis, and eutopic endometrium from healthy women to create a single-cell transcriptome atlas of endometriosis.

Results: We have identified 9 cell types and performed single-cell analysis of fibroblasts, and determined a potential developmental trajectory associated with endometriosis. We also identified fibroblast subpopulations related to endometriosis development and found that *StAR* played an important role in this process. Moreover, T cells in endometriosis were less activated or inflammatory with decreased effector CD8 + T cells, while the composition ratio of natural killer cells decreased and the percentage of monocytes/macrophages increased in endometriosis cysts. In addition, the effectiveness of immune cells in endometriosis lesions, eutopic endometrium from women with endometriosis, and eutopic endometrium from healthy women was distinct. Cell–cell interaction analyses highlighted the imbalanced immune environment in endometriosis lesions and immune cells in endometriosis could promote the development of the disease.

Conclusion: Our study provided a systematic characterisation of endometriosis and insights into the aetiology and pathology of endometriosis.

Keywords: Endometriosis, Single-cell sequence, Fibroblast, Immune cell heterogeneity



Original research

Topological analysis of hepatocellular carcinoma tumour microenvironment based on imaging mass cytometry reveals cellular neighbourhood regulated reversely by macrophages with different ontogeny

Jianpeng Sheng ,[1,2,3] Junlei Zhang,[1,2] Lin Wang,[2] Vincent Tano,[4] Jianghui Tang,[1,2] Xun Wang,[1,2] Jiangchao Wu,[1,2] Jinyuan Song,[1,2] Yaxing Zhao,[2] Jingxia Rong,[2] Fei Cheng,[5] Jianfeng Wang,[1,2] Yinan Shen,[1,2] Liang Wen,[1,2] Junjun He,[2] Hui Zhang,[2] Taohong Li,[2] Qi Zhang,[1,2,3] Xueli Bai ,[1,2,3] Zhimin Lu,[1,3,6] Tingbo Liang[1,2,3]

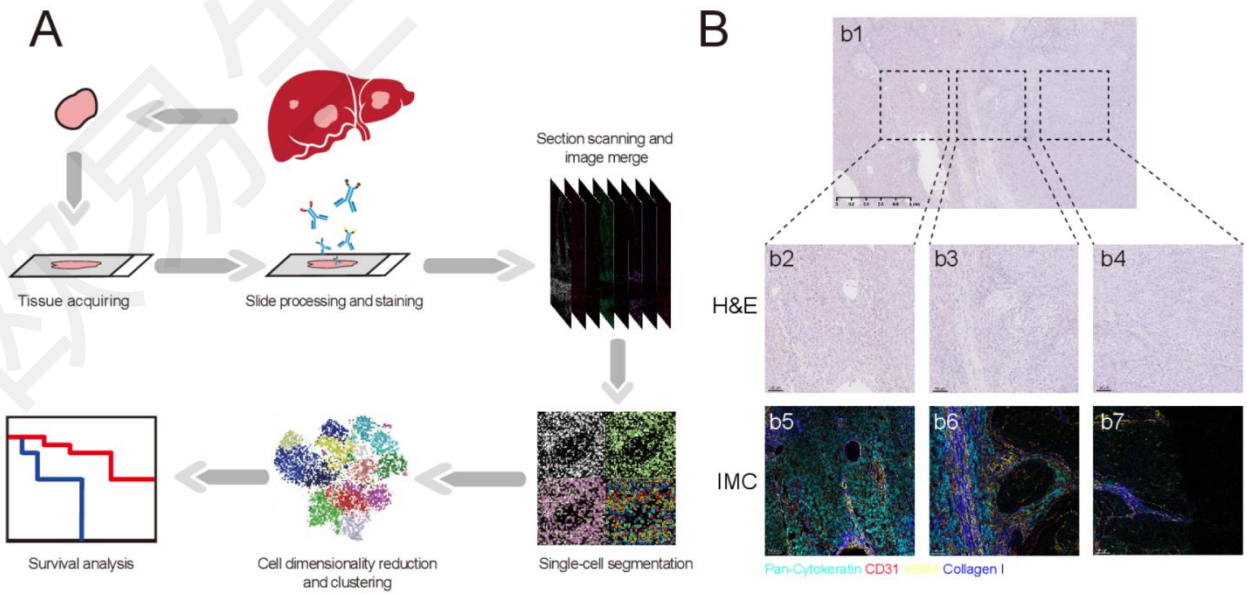
ABSTRACT

Objective Hepatocellular carcinoma (HCC) tumour microenvironment (TME) is highly complex with diverse cellular components organising into various functional units, cellular neighbourhoods (CNs). And we wanted to define CN of HCC while preserving the TME architecture, based on which, potential targets for novel immunotherapy could be identified.

Design A highly multiplexed imaging mass cytometry (IMC) panel was designed to simultaneously quantify 36 biomarkers of tissues from 134 patients with HCC and 7 healthy donors to generate 562 highly multiplexed histology images at single-cell resolution. Different function units were defined by topological analysis of TME. CN relevant to the patients' prognosis was identified as specific target for HCC therapy. Transgenic mouse models were used to validate the novel immunotherapy target for HCC.

Results Three major types of intratumour areas with distinct distribution patterns of tumorous, stromal and immune cells were identified. 22 cellular metaclusters and 16 CN were defined. CN composed of various types of cells formed regional function units and the regional immunity was regulated reversely by resident Kupffer cells and infiltrating macrophages with protumour and antitumour function, respectively. Depletion of Kupffer cells in mouse liver largely enhances the T cell response, reduces liver tumour growth and sensitises the tumour response to antiprogrammed cell death protein-1 treatment.

Conclusion Our findings reveal for the first time the various topological function units of HCC TME, which also presents the largest depository of pathological landscape for HCC. This work highlights the potential of Kupffer cell-specific targeting rather than overall myeloid cell blocking as a novel immunotherapy for HCC treatment.



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DOI: 10.1002/ctm2.500

CLINICAL AND TRANSLATIONAL MEDICINE Open Access WILEY

RESEARCH ARTICLE

Single-cell transcriptomes reveal heterogeneity of high-grade serous ovarian carcinoma

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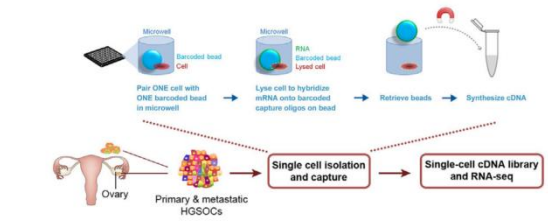
Abstract

Background: High-grade serous ovarian carcinoma (HGSOC) is the most com- mon and aggressive histotype of epithelial ovarian cancer. The heterogeneity and molecular basis of this disease remain incompletely understood.

Methods: To address this question, we have performed a single-cell transcrip- tomics analysis of matched primary and metastatic HGSOC samples.

Results: A total of 13 571 cells are categorized into six distinct cell types, includ- ing epithelial cells, fibroblast cells, T cells, B cells, macrophages, and endothe- lial cells. A subset of aggressive epithelial cells with hyperproliferative and drug- resistant potentials is identified.

Several new markers that are highly expressed in epithelial cells are characterized, and their roles in ovarian cancer cell growth and migration are further confirmed. Dysregulation of multiple signaling path- ways, including the translational machinery, is associated with ovarian cancer metastasis through the trajectory analysis. Moreover, single-cell regulatory net- work inference and clustering (SCENIC) analysis reveals the gene regulatory net- works and suggests the JUN signaling pathway as a potential therapeutic target for treatment of ovarian cancer, which is validated using the JUN/AP-1 inhibitor T-5224. Finally, our study depicts the epithelial-fibroblast cell communication atlas and identifies several important receptor-ligand complexes in ovarian can- cer development.



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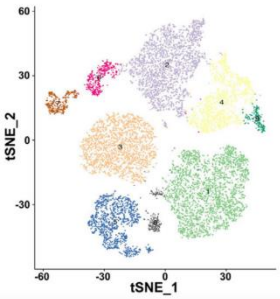

Cell type	Cell number	Percentage
1. Epithelial cell	3417	25.2 %
2. Fibroblast-I	2963	21.8 %
3. T cell	2569	18.9 %
4. Fibroblast-II	1637	12.1 %
5. Macrophage	1505	11.1 %
6. Fibroblast-III	504	3.7 %
7. Endothelial cell	421	3.1 %
8. B cell	288	2.1 %
9. Fibroblast-IV	267	2.0 %

frontiers in Oncology

ORIGINAL RESEARCH
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Single-Cell Transcriptomics of Glioblastoma Reveals a Unique Tumor Microenvironment and Potential Immunotherapeutic Target Against Tumor-Associated Macrophage

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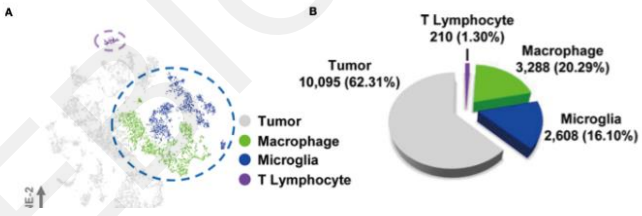
Background: The main immune cells in GBM are tumor-associated macrophages (TAMs). Thus far, the studies investigating the activation status of TAM in GBM are mainly limited to bulk RNA analyses of individual tumor biopsies. The activation states and transcriptional signatures of TAMs in GBM remain poorly characterized.

Methods: We comprehensively analyzed single-cell RNA-sequencing data, covering a total of 16,201 cells, to clarify the relative proportions of the immune cells infiltrating GBMs. The origin and TAM states in GBM were characterized using the expression profiles of differential marker genes. The vital transcription factors were examined by SCENIC analysis. By comparing the variable gene expression patterns in different clusters and cell types, we identified components and characteristics of TAMs unique to each GBM subtype. Meanwhile, we interrogated the correlation between SPI1 expression and macrophage infiltration in the TCGA-GBM dataset.

Results: The expression patterns of TMEM119 and MHC-II can be utilized to distinguish the origin and activation states of TAMs. In TCGA-Mixed tumors, almost all TAMs were bone marrow-derived macrophages. The TAMs in TCGA-proneural tumors were characterized by primed microglia. A different composition was observed in TCGA-classical tumors, which were infiltrated by repressed microglia. Our results further identified SPI1 as a crucial regulon and potential immunotherapeutic target important for TAM maturation and polarization in GBM.

Conclusions: We describe the immune landscape of human GBM at a single-cell level and define a novel categorization scheme for TAMs in GBM. The immunotherapy against



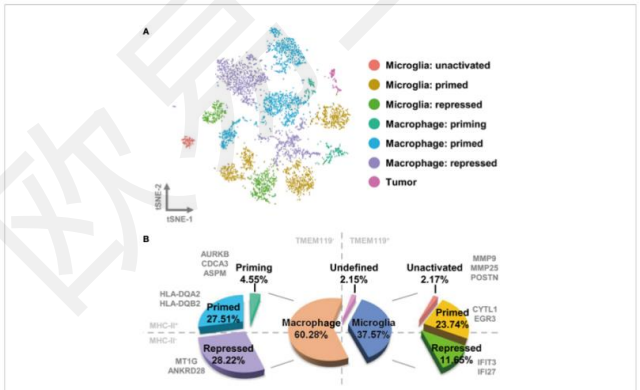


FIGURE 2 | TAMs in GBM comprise different activation states of bone marrow–derived macrophages and brain-resident microglia. **(A)** Each cell type is visualized as a t-SNE plot. **(B)** The relative proportions of macrophages and microglia are 60.28% and 37.57%, respectively. The ratio of detailed TAM states is shown in the pie chart.

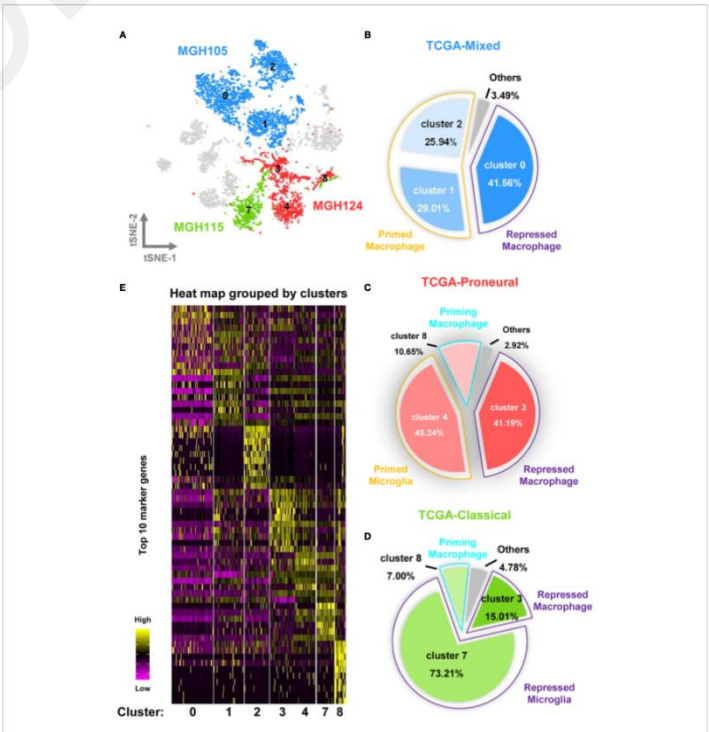


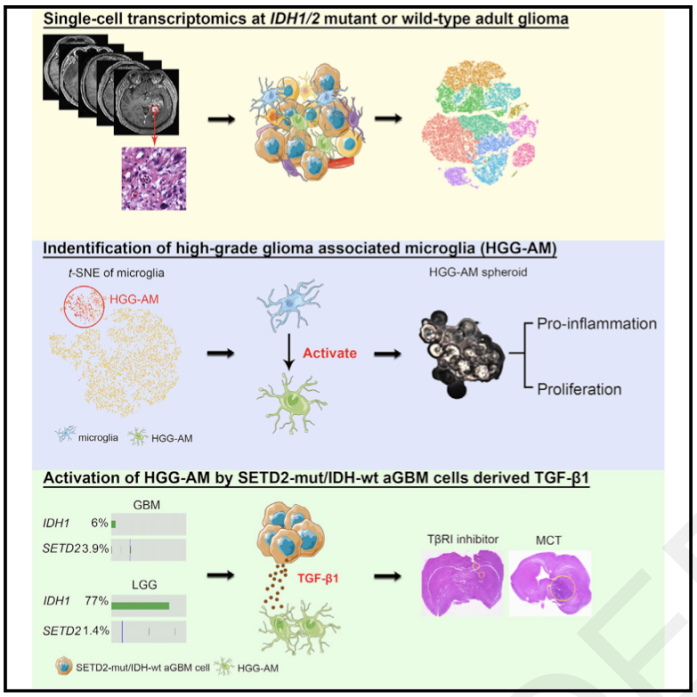
FIGURE 3 | Different molecular subtypes of GBM have distinct TAM composition and characteristics. **(A)** The cell distribution of MGH105, MGH115, and MGH124 are shown by the t-SNE plot. **(B)** TAMs in the TCGA-mixed tumor comprise repressed macrophages, primed macrophages, and others. **(C)** TAMs in the TCGA-proneural tumor comprise repressed macrophage, primed microglia, priming macrophages, and others. **(D)** TAMs in the TCGA-classical tumor contain repressed macrophages, repressed microglia, priming macrophages, and others. **(E)** The expression profiles of the top 10 marker genes of each cluster are displayed as a heat map.

Article

Cell Reports

Pro-inflammatory and proliferative microglia drive progression of glioblastoma

Graphical abstract



Authors

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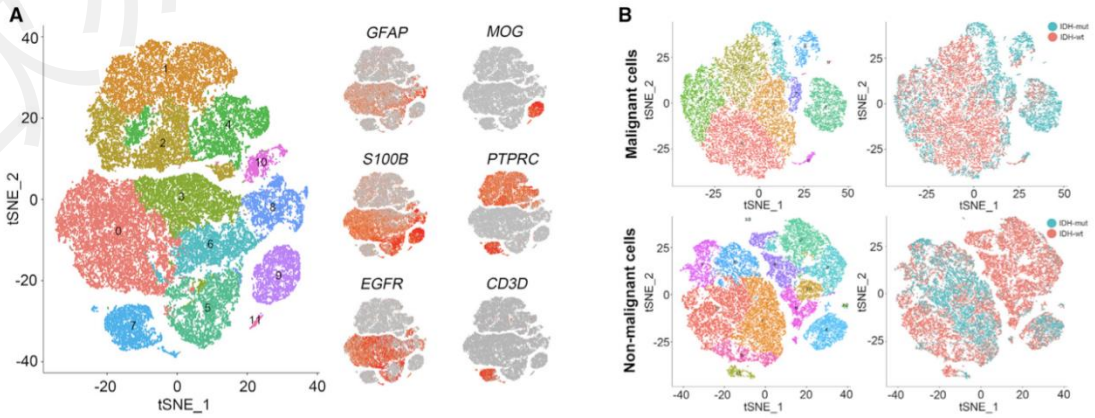
In brief

Liu et al. find that high-grade glioma-associated microglia exhibit inflammasome-mediated pro-inflammatory and proliferative signatures shaping the cytokine microenvironment to promote oncogenesis.

SUMMARY

Scant understanding of the glioblastoma microenvironment and molecular bases hampers development of efficient treatment strategies. Analyses of gene signatures of human gliomas demonstrate that the *SETD2* mutation is correlated with poor prognosis of *IDH1/2* wild-type (IDH-WT) adult glioblastoma patients. To better understand the crosstalk between *SETD2* mutant (SETD2-mut) glioblastoma cells and the tumor microenvironment, we leverage single-cell transcriptomics to comprehensively map cellular populations in glioblastoma. In this study, we identify a specific subtype of high-grade glioma-associated microglia (HGG-AM). Further analysis shows that transforming growth factor (TGF)-β1 derived from SETD2-mut/IDH-WT tumor cells activates HGG-AM, exhibiting pro-inflammation and proliferation signatures. Particularly, HGG-AM secretes interleukin (IL)-1β via the apolipoprotein E (ApoE)-mediated NLRP1 inflammasome, thereby promoting tumor progression. HGG-AM present extensive proliferation and infiltration to supplement the activated microglia pool. Notably, TGF-β1/TβRI depletion dramatically reduces HGG-AM density and suppresses tumor growth. Altogether, our studies identify a specific microglia subpopulation and establish the cellular basis of interactions between HGG-AM and glioblastoma cells.



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Research Paper

Visualization of endogenous p27 and Ki67 reveals the importance of a c-Myc-driven metabolic switch in promoting survival of quiescent cancer cells

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Abstract

Rationale: Recurrent and metastatic cancers often undergo a period of dormancy, which is closely associated with cellular quiescence, a state whereby cells exit the cell cycle and are reversibly arrested in G0 phase. Curative cancer treatment thus requires therapies that either sustain the dormant state of quiescent cancer cells, or preferentially, eliminate them. However, the mechanisms responsible for the survival of quiescent cancer cells remain obscure.

Methods: Dual genome-editing was carried out using a CRISPR/Cas9-based system to label endogenous p27 and Ki67 with the green and red fluorescent proteins EGFP and mCherry, respectively, in melanoma cells. Analysis of transcriptomes of isolated EGFP-p27^{high}mCherry-Ki67^{low} quiescent cells was conducted at bulk and single cell levels using RNA-sequencing. The extracellular acidification rate and oxygen consumption rate were measured to define metabolic phenotypes. SiRNA and inducible shRNA knockdown, chromatin immunoprecipitation and luciferase reporter assays were employed to elucidate mechanisms of the metabolic switch in quiescent cells.

Results: Dual labelling of endogenous p27 and Ki67 with differentiable fluorescent probes allowed for visualization, isolation, and analysis of viable p27^{high}Ki67^{low} quiescent cells. Paradoxically, the proto-oncoprotein c-Myc, which commonly drives malignant cell cycle progression, was expressed at relatively high levels in p27^{high}Ki67^{low} quiescent cells and supported their survival through promoting mitochondrial oxidative phosphorylation (OXPHOS). In this context, c-Myc selectively transactivated genes encoding OXPHOS enzymes, including subunits of isocitric dehydrogenase 3 (IDH3), whereas its binding to cell cycle progression gene promoters was decreased in quiescent cells. Silencing of c-Myc or the catalytic subunit of IDH3, IDH3α, preferentially killed quiescent cells, recapitulating the effect of treatment with OXPHOS inhibitors.

Conclusion: These results establish a rigorous experimental system for investigating cellular quiescence, uncover the high selectivity of c-Myc in activating OXPHOS genes in quiescent cells, and propose OXPHOS targeting as a potential therapeutic avenue to counter cancer cells in quiescence.

Key words: c-Myc, IDH3, quiescence, quiescent cells, oxidative phosphorylation

Oncogenesis www.nature.com/oncsis

ARTICLE OPEN

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Dissecting the single-cell transcriptome network in patients with esophageal squamous cell carcinoma receiving operative paclitaxel plus platinum chemotherapy

Zhencong Chen[1,3], Yiwei Huang[1,3], Zhengyang Hu[1,3], Mengnan Zhao[1,3], Yunyi Bian[1], Zongwei Chen[1], Yuansheng Zheng[1], Guoshu Bi[1], Yanrui Pang[2], Cheng Zhan[1✉], Zongwu Lin[1✉], Weigang Guo[1✉], Qun Wang[1] and Lijie Tan[1]

Esophageal squamous cell carcinoma (ESCC) accounts for 90% of all cases of esophageal cancers worldwide. Although neoadjuvant chemotherapy (NACT-ESCC) improves the survival of ESCC patients, the five-year survival rate of these patients is dismal. The tumor microenvironment (TME) and tumor heterogeneity decrease the efficacy of ESCC therapy. In our study, 113,581 cells obtained from five ESCC patients who underwent surgery alone (SA-ESCC) and five patients who underwent preoperative paclitaxel plus platinum chemotherapy (NACT-ESCC), were used for scRNA-seq analysis to explore molecular and cellular reprogramming patterns. The results showed samples from NACT-ESCC patients exhibited the characteristics of malignant cells and TME unlike samples from SA-ESCC patients. Cancer cells from NACT-ESCC samples were mainly at the 'intermediate transient stage'. Stromal cell dynamics showed molecular and functional shifts that formed the immune-activation microenvironment. APOE, APOC1, and SPP1 were highly expressed in tumor-associated macrophages resulting in anti-inflammatory macrophage phenotypes. Levels of CD8+ T cells between SA-ESCC and NACT-ESCC tissues were significantly different. Immune checkpoints analysis revealed that LAG3 is a potential immunotherapeutic target for both NACT-ESCC and SA-ESCC patients. Cell–cell interactions analysis showed the complex cell-cell communication networks in the TME. In summary, our findings elucidate on the molecular and cellular reprogramming of NACT-ESCC and ESCC patients. These findings provide information on the potential diagnostic and therapeutic targets for ESCC patients.

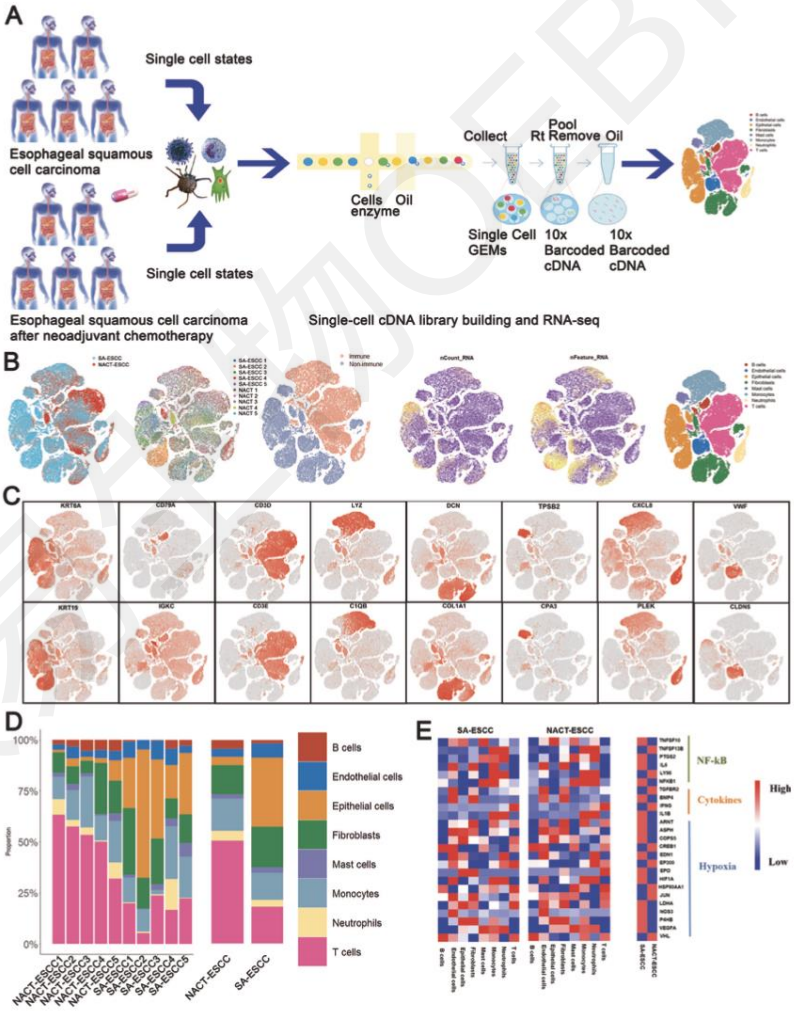

Fig. 1 A Single-Cell Atlas of SA-ESCC and NACT-ESCC. A The workflow showing the collection and processing of specimens from SA-ESCC and NACT-ESCC tissues for scRNA-seq analysis. **B** TSNE of 113,581 cells, each cell has a color code. From left to right are: origin of sample type (SA-ESCC or NACT-ESCC), the corresponding patient, immune type, transcript counts, transcript features, and associated cell type. **C** Expression of marker genes for each cell subtype. **D** The proportion of each cell type in SA-ESCC and NACT-ESCC samples. **E** Heatmap of representative genes in cytokines, nuclear factor-kB (NF-kB), and hypoxia signaling pathways mapped onto cell types in SA-ESCC and NACT-ESCC samples.

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The histone demethylase Kdm6b regulates the maturation and cytotoxicity of TCRαβ^{+}CD8αα^{+} intestinal intraepithelial lymphocytes

Haohao Zhang[1,2,8], Yiming Hu[1,2,8], Dandan Liu[1,2,8], Zhi Liu[1,2], Ningxia Xie[1], Sanhong Liu[3], Jie Zhang[2], Yuhang Jiang[1,2], Cuifeng Li[1,2], Qi Wang[1,2], Xi Chen[2], Deji Ye[1,2], Donglin Sun[1], Yujia Zhai[2], Xinhui Yan[2], Yongzhong Liu[4], Charlie Degui Chen[5], Xingxu Huang[1], Y. Eugene Chin[6], Yufang Shi[2,6], Baojin Wu[7]✉ and Xiaoren Zhang[1,2]✉

Intestinal intraepithelial lymphocytes (IELs) are distributed along the length of the intestine and are considered the frontline of immune surveillance. The precise molecular mechanisms, especially epigenetic regulation, of their development and function are poorly understood. The trimethylation of histone 3 at lysine 27 (H3K27Me3) is a kind of histone modifications and associated with gene repression. Kdm6b is an epigenetic enzyme responsible for the demethylation of H3K27Me3 and thus promotes gene expression. Here we identified Kdm6b as an important intracellular regulator of small intestinal IELs. Mice genetically deficient for Kdm6b showed greatly reduced numbers of TCRαβ^{+}CD8αα^{+} IELs. In the absence of Kdm6b, TCRαβ^{+}CD8αα^{+} IELs exhibited increased apoptosis, disturbed maturation and a compromised capability to lyse target cells. Both IL-15 and Kdm6b-mediated demethylation of histone 3 at lysine 27 are responsible for the maturation of TCRαβ^{+}CD8αα^{+} IELs through upregulating the expression of Gzmb and Fasl. In addition, Kdm6b also regulates the expression of the gut-homing molecule CCR9 by controlling H3K27Me3 level at its promoter. However, Kdm6b is dispensable for the reactivity of thymic precursors of TCRαβ^{+}CD8αα^{+} IELs (IELPs) to IL-15 and TGF-β. In conclusion, we showed that Kdm6b plays critical roles in the maturation and cytotoxic function of small intestinal TCRαβ^{+}CD8αα^{+} IELs.

Cell Death & Differentiation; https://doi.org/10.1038/s41418-021-00921-w

Figure 1

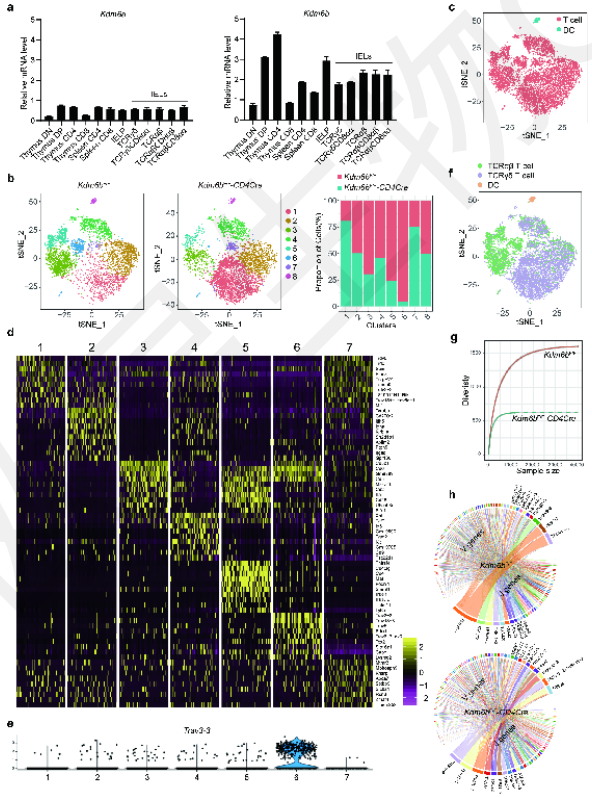
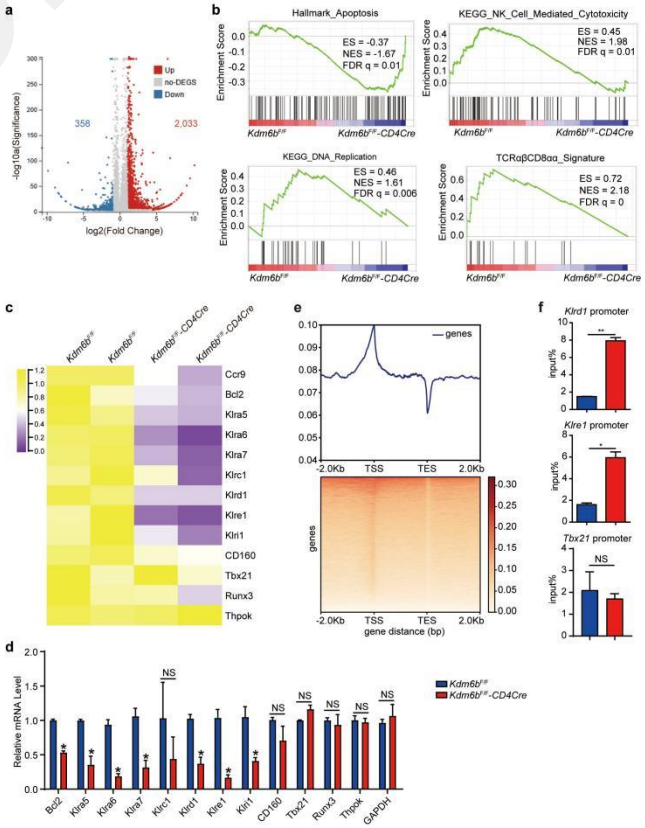


Figure 5



frontiers

A case study of relapsed and refractory multiple myeloma reveals clonal evolution and gene regulatory networks of plasma cells via combining consecutive genomics with single-cell transcriptomes

Peng Liu[1*], Jiadai Xu[1], Yue Wang[1], Zheng Wei[1], Jingli Zhuang[1], Jing Li[1], Yifeng Sun[1], Panpan Li[1], Shiyang Gu[1], Yian Zhang[1], Jifeng Jiang[1], Liang Ren[1], Yawen Wang[1], Chen Chen[1], Yu Zhang[2]

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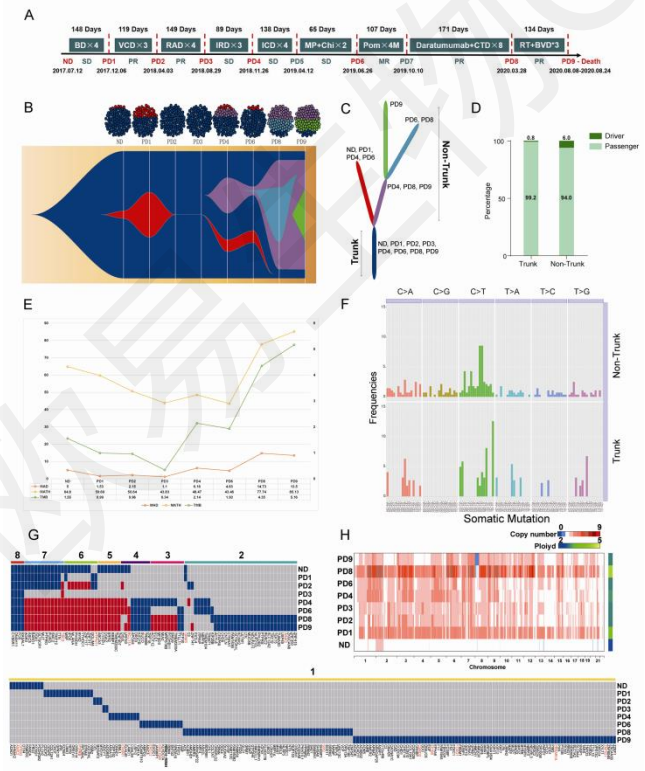
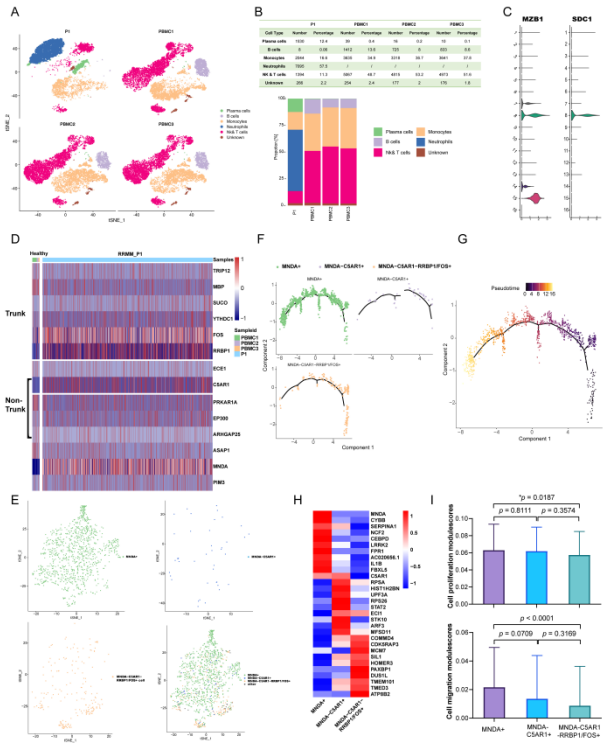
- **Abstract**

This study attempted to investigate how clonal structure evolves, along with potential regulatory networks, as a result of multiline therapies in relapsed/refractory multiple myeloma (RRMM). Eight whole exome sequencing (WES) and one single cell RNA sequencing (scRNA-seq) were performed in order to assess dynamic genomic changes in temporal consecutive samples of one RRMM patient from the time of diagnosis to death (about 37 months). The 63-year-old female patient who suffered from MM (P1) had disease progression (PD) nine times from July 2017 (newly diagnosed (ND)) to Aug 2020 (death), and the force to drive branching-pattern evolution of malignant PCs was found to be sustained. The mutant-allele tumor heterogeneity (MATH) and tumor mutation burden (TMB) initially exhibited a downward trend, which was then upward throughout the course of the disease. Various somatic single nucleotide variants (SNVs) that had disappeared after the previous treatment were observed to reappear in later stages. Chromosomal instability (CIN) and homologous recombination deficiency (HRD) scores were observed to be increased during periods of all progression, especially in the period of extramedullary plasmacytoma. Finally, in combination with WES and scRNA-seq of P1-PD9 (the nineth PD), the intro-heterogeneity and gene regulatory networks of MM cells were deciphered. As verified by the overall survival of MM patients in the MMRF CoMMpass and GSE24080 datasets, RUNX3 was identified as a potential driver for RRMM.





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BMC Medicine

RESEARCH ARTICLE Open Access

5mC regulator-mediated molecular subtypes depict the hallmarks of the tumor microenvironment and guide precision medicine in bladder cancer

Jiao Hu[1], Belaydi Othmane[1], Anze Yu[1,2], Huihuang Li[1], Zhiyong Cai[1], Xu Chen[1,3], Wenbiao Ren[1,4], Jinbo Chen[1*] and Xiongbing Zu[1*]

Abstract

Background: Depicting the heterogeneity and functional characteristics of the tumor microenvironment (TME) is necessary to achieve precision medicine for bladder cancer (BLCA). Although classical molecular subtypes effectively reflect TME heterogeneity and characteristics, their clinical application is limited by several issues.

Methods: In this study, we integrated the Xiangya cohort and multiple external BLCA cohorts to develop a novel 5-methylcytosine (5mC) regulator-mediated molecular subtype system and a corresponding quantitative indicator, the 5mC score. Unsupervised clustering was performed to identify novel 5mC regulator-mediated molecular subtypes. The principal component analysis was applied to calculate the 5mC score. Then, we correlated the 5mC clusters (5mC score) with classical molecular subtypes, immunophenotypes, clinical outcomes, and therapeutic opportunities in BLCA. Finally, we performed pancancer analyses on the 5mC score.

Results: Two 5mC clusters, including 5mC cluster 1 and cluster 2, were identified. These novel 5mC clusters (5mC score) could accurately predict classical molecular subtypes, immunophenotypes, prognosis, and therapeutic opportunities of BLCA. 5mC cluster 1 (high 5mC score) indicated a luminal subtype and noninflamed phenotype, characterized by lower anticancer immunity but better prognosis. Moreover, 5mC cluster 1 (high 5mC score) predicted low sensitivity to cancer immunotherapy, neoadjuvant chemotherapy, and radiotherapy, but high sensitivity to antiangiogenic therapy and targeted therapies, such as blocking the β-catenin, FGFR3, and PPAR-γ pathways.

Conclusions: The novel 5mC regulator-based subtype system reflects many aspects of BLCA biology and provides new insights into precision medicine in BLCA. Furthermore, the 5mC score may be a generalizable predictor of immunotherapy response and prognosis in pancancers.

Keywords: Bladder cancer, 5-Methylcytosine, Molecular subtype, Tumor microenvironment, Immune phenotype, Immunotherapy

Radiation Medicine and Protection 2 (2021) 181–183

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Letter

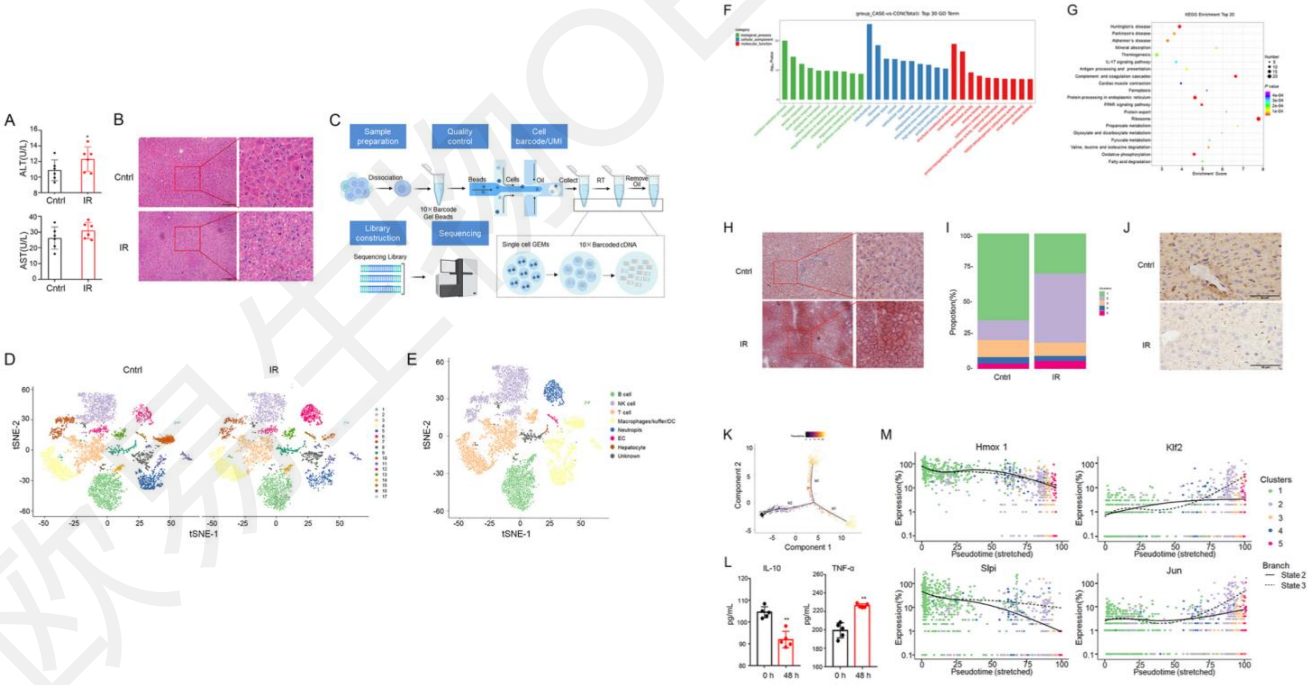
Single-cell RNA sequencing reveals the cell landscape of a radiation-induced liver injury mouse model

ARTICLE INFO

Keywords
Radiation induced liver injury
Single-cell RNA sequencing
Hepatocyte
Macrophage

ABSTRACT

The mechanisms of radiation-induced liver injury (RILI) has not been fully elucidated so far. In the present study, a RILI mouse model was constructed by exposing the liver to a single dose of 30 Gy X-rays. Liver injuries consisting of liver function damage and histopathological variations were confirmed after 2 weeks. And then the cellular atlas of RILI liver was generated by profiling 9,641 cells isolated from X-ray irradiated mice livers and control ones from RILI mice model using single-cell RNA sequencing (scRNA-seq). Seven cell types were identified, including B cells, natural killer cells, T cells, macrophages/Küpffer cells/Dendritic cells (DC), neutrophils, endothelial cells, and hepatocyte. Although there was no significant difference of overall cell typing was observed between the Control and RILI groups, hepatocytes and macro/Küpffer/DC cell types were chosen for further functional exploration. Gene expression profiles and bioinformatics analysis of hepatocytes revealed that multiple metabolic related pathways were enriched in livers exposed to IR. These scRNA-seq data were confirmed in RILI liver samples via adipose staining. Besides, obviously varied M1-/M2-macrophages polarization was observed in RILI liver, which was in accordance with the enzyme linked immunosorbent assay (ELISA) results of IR-induced M2 to pro-inflammatory M1 macrophages transformation in mouse macrophage cell line Raw264.7. In addition, we predicted that several genes were found to differentially expressed during the process of macrophage polarization from M2 to M1 subtype. Overall, our study provides a cellular landscape of RILI at single-cell resolution that indicates the characteristics of hepatocytes and macrophages, which will contribute to investigate the novel therapeutic or preventive management for RILI.



Research Article | Deconstructing Organs: Single-Cell Analyses, Decellularized Organs, Organoids, and Organ-on-a-Chip Models

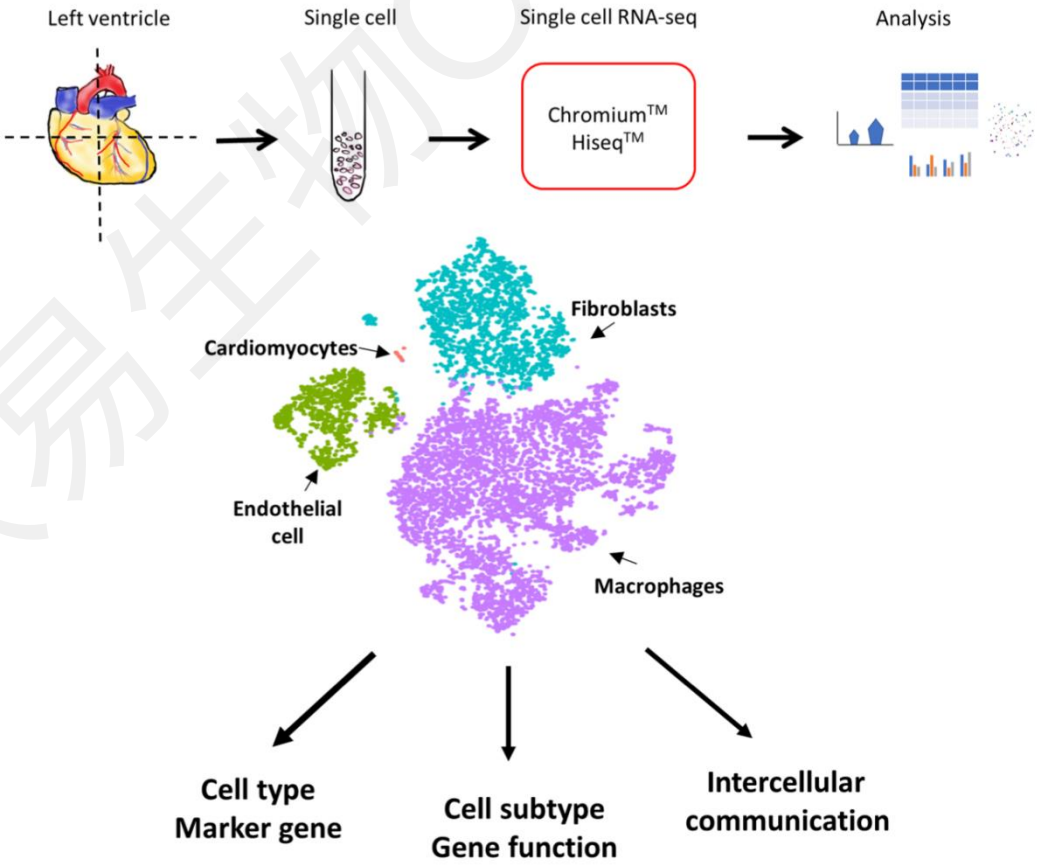
Single-cell RNA sequencing of mouse left ventricle reveals cellular diversity and intercommunication

Lan Wu ,* Yan-Fei Li,* Jun-Wei Shen,* Qian Zhu, Jing Jiang, Shi-Hua Ma, Kai He, Zhong-Ping Ning, Jue Li, and Xin-Ming Li Show fewer authors

21 DEC 2021 // https://doi.org/10.1152/physiolgenomics.00016.2021

ABSTRACT

Previous studies have revealed the diversity of the whole cardiac cellulome but not refined the left ventricle, which was essential for finding therapeutic targets. Here, we characterized single-cell transcriptional profiles of the mouse left ventricular cellular landscape using single-cell RNA sequencing (10×Genomics). Detailed t-Distributed Stochastic Neighbor Embedding (tSNE) analysis revealed the cell types of left ventricle with gene markers. Left ventricular cellulome contained cardiomyocytes highly expressed Trdn, endothelial cells highly expressed Pcdh17, fibroblast highly expressed Lama2 and macrophages highly expressed Hpgds, also proved by in situ hybridization. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analysis (ListHits>2, $p<0.05$) were employed with the DAVID database to investigate subtypes of each cell type with the underlying functions of differentially expressed genes (DEGs). Endothelial cells included five subtypes, fibroblasts comprised of seven subtypes and macrophages contained eleven subtypes. The key representative DEGs ($p<0.001$) were Gja4 and Gja5 in cluster 3 of endothelial cells, Aqp2 and Thbs4 in cluster 2 of fibroblasts, as well as Clec4e and Trem-1 in in cluster 3 of marcophages perhaps involved in the occur of atherosclerosis, heart failure and acute myocardial infarction proved by literature review. We also revealed extensive networks of intercellular communication in left ventricle. We suggested possible therapeutic targets for cardiovascular disease and autocrine and paracrine signaling underpins left ventricular homeostasis. This study provided new insights into the structure and function of the mammalian left ventricular cellulome and offers an important resource that will stimulate studies in cardiovascular research.



JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Islet β-cells physiological difference study of old and young mice based on single-cell transcriptomics

Zeyu Zheng[1], Qiufeng Zhan[1], Ayun Chen[1], Zhen Yu[2], Gang Chen[1,2,3,*]

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ABSTRACT

Aims/Introduction: Body aging is a universal biological process. With aging, cells undergo a series of physiological changes. The main feature is cell proliferation decline, although the cells still have normal functions. Pancreatic β-cells are no exception. However, the physiological senescence of β-cells, and the resulting function and transcriptome changes have rarely attracted attention. The specific senescence phenotype of β-cells remains unknown.

Materials and Methods: Pancreatic samples from three female C57BL/6 mice with aged 2.5 months (young) mice and 20 months (old) were digested to a single-cell suspension and analyzed, with 10× Genomics single-cell ribonucleic acid sequencing, β-cells were determined by biosynthesis analysis, and differences between old and young mice were identified.

Results: A total of 47 differential genes with significant and statistical significance were screened in β-cells (fold change >1.5, $P < 0.05$). In old mice, 27 genes were upregulated and 20 genes were downregulated. Genes *Mt1*, *Mt2*, *Pyy*, *Gcg* and *Pnlip*, and mitochondrial genes *mt-Nd1*, *mt-Nd3*, *mt-Co1*, *mt-Co2* and *mt-Co3* were found to be involved in cellular senescence. Transcription factors *Jund* and *Fos* were important regulators of senescence.

Conclusions: An overall difference was found between the pancreatic β-cells of old and young mice. Transcription factors facilitate transitions between pancreatic β-cells. These findings are worthy of deep exploration, and provide new resources and directions for the research of pancreatic aging in mice.

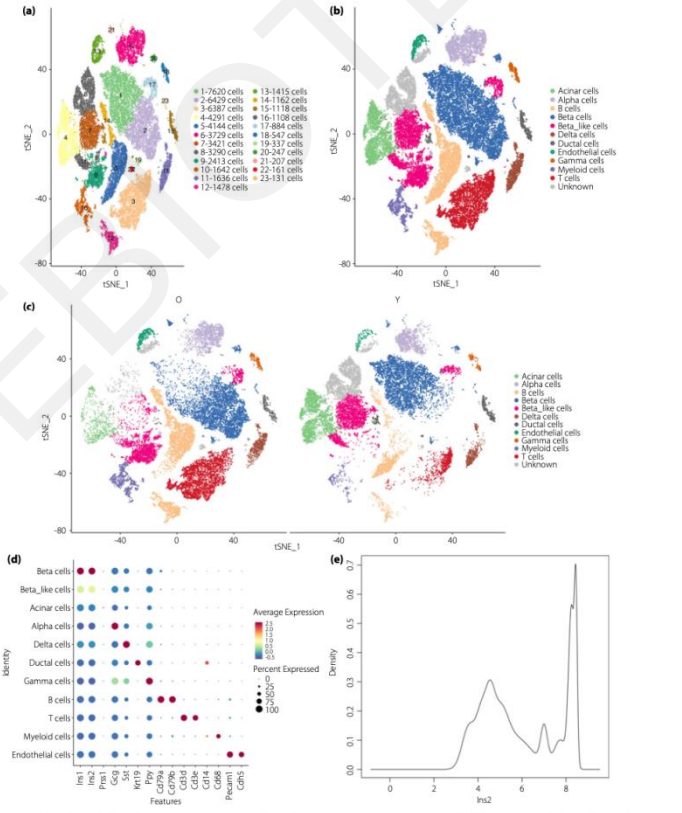

Figure 1 | Integrated single-cell ribonucleic acid sequencing analysis of young mice pancreatic cells and old pancreatic cells. (a) Cells on the t-distributed stochastic neighbor embedding plot of 23 clusters. (b) Cellular populations identified. (c) Cells on the t-distributed stochastic neighbor embedding plot of all six samples were colored as originating either from young mice pancreatic cells and old pancreatic cells. (d) Canonical cell markers were used to label clusters by cell identity as represented in the t-distributed stochastic neighbor embedding plot. Cell types were classified as β-cells (C1, C2, C19, C20, C21, C23), β-like-cells (C7, C9, C16, C17), α-cells (C6), γ-cells (C18), δ-cells (C11), acinar cells, ductal cells (C15), endothelial cells (C13), T cells (C3), B cells (C5, C12, C14) and myeloid cells (C10). (e) Bimodal distribution of Ins2 expression.

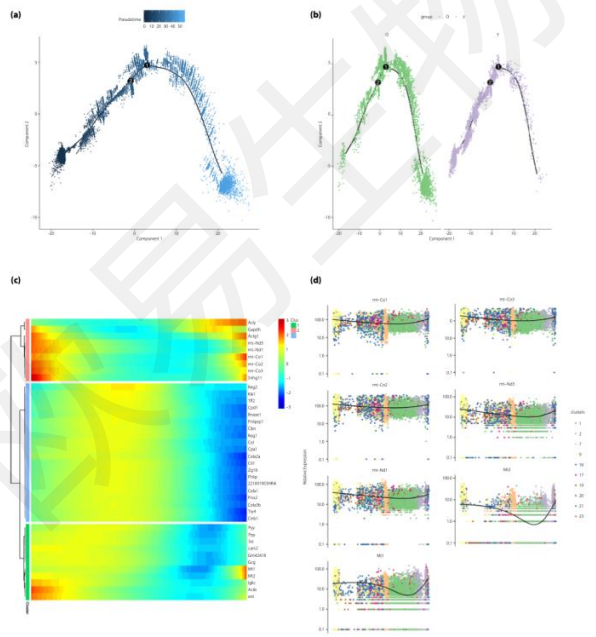

Figure 3 | Pseudotime analysis of the old mice β-cells and young mice β-cells. Each dot represents one cell. (a,b) *In silico* pseudotime ordering of β-cells shows that 'young β-cells' are along the main path of the left state. Whereas 'old β-cells' are along the right state. (c) Clustering of differentially modulated genes by pseudotime progression of β-cells shows distinct kinetics of gene responses to cell conversion. (d) Gene expression kinetics along pseudotime progression of representative genes.

nature COMMUNICATIONS

ARTICLE

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Stroke subtype-dependent synapse elimination by reactive gliosis in mice

Xiaojing Shi[1,6], Longlong Luo[1,5,6], Jixian Wang[2,6], Hui Shen[1], Yongfang Li[2], Muyassar Mamtilahun[1], Chang Liu[1], Rubing Shi[1], Joon-Hyuk Lee[3], Hengli Tian[1], Zhijun Zhang[1], Yongting Wang[1], Won-Suk Chung[3✉], Yaohui Tang[1✉] & Guo-Yuan Yang[1,4✉]

The pathological role of reactive gliosis in CNS repair remains controversial. In this study, using murine ischemic and hemorrhagic stroke models, we demonstrated that microglia/ macrophages and astrocytes are differentially involved in engulfing synapses in the reactive gliosis region. By specifically deleting MEGF10 and MERTK phagocytic receptors, we deter- mined that inhibiting phagocytosis of microglia/macrophages or astrocytes in ischemic stroke improved neurobehavioral outcomes and attenuated brain damage. In hemorrhagic stroke, inhibiting phagocytosis of microglia/macrophages but not astrocytes improved neu- robehavioral outcomes. Single-cell RNA sequencing revealed that phagocytosis related bio- logical processes and pathways were downregulated in astrocytes of the hemorrhagic brain compared to the ischemic brain. Together, these findings suggest that reactive microgliosis and astrogliosis play individual roles in mediating synapse engulfment in pathologically dis- tinct murine stroke models and preventing this process could rescue synapse loss.

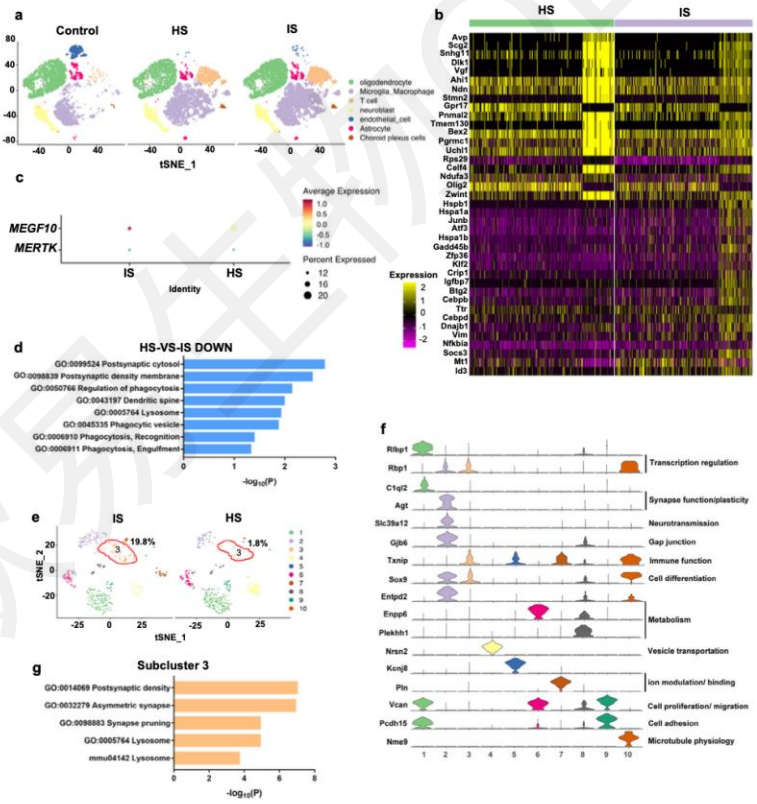

Fig. 10 scRNA-seq revealed phagocytosis-related gene expression difference of astrocytes between ischemic and hemorrhagic stroke. **a** tSNE map showed the expression profiles of the striatum in control, ischemic and hemorrhagic mice, respectively. **b** Heatmap showed fold change of top 20 genes. **c** Dot plot showing MEGF10 and MERTK expression in astrocytes. **d** Bar chart showed phagocytosis-related GO and KEGG pathways that downregulated in hemorrhagic stroke (HS), as compared with ischemic stroke (IS). Representative terms were shown in rows and $-\log_{10}(p)$ in columns. **e** tSNE map showed subclusters of astrocytes in IS and HS. **f** Violin plots showed top marker genes in specific astrocyte subclusters. **g** Bar chart showed phagocytosis-related GO and KEGG terms enriched in subcluster 3 of astrocytes.

frontiers in Genetics

ORIGINAL RESEARCH
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Single-Cell RNA-Seq of Bone Marrow Cells in Aplastic Anemia

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Aplastic anemia (AA) is an autoimmune disease characterized by peripheral blood pancytopenia and bone marrow failure. Recently, a research study verified bone marrow failure of AA patients resulting from hematopoietic stem and progenitor cell (HSPC) attack by active T cells. Nonetheless, whether B cells, as one of the important immune cells, destruct the hematopoiesis is still unclear. Here, a large-scale single-cell transcriptomic sequencing of 20,000 bone marrow cells from AA patients and healthy donors was performed. A total of 17 clusters and differentially expressed genes were identified in each cluster relative to other clusters, which were considered potential marker genes in each cluster. The top differentially expressed genes in HSPCs (S100A8, RETN, and TNFAIP3), monocytes (CXCL8, JUN, and IL1B), and neutrophils and granulocytes (CXCL8, NFKBIA, and MT-CYB) were related to immune and inflammatory injury. Then, the B-cell receptor (BCR) diversities and pairing frequencies of V and J genes were analyzed. The highest pairing frequencies in AA patients were IGHV3-20-IGKJ2, IGHV3-20-IGKJ4, and IGHV3-20-IGHLJ2. Meanwhile, there were 3 V genes, including IGHV3-7, IGHV3-33, and IGLV2-11, with elevated expression in B cells from AA patients. Cell type–specific ligand–receptor was further identified in B-cell interaction with hematopoietic cells in the bone marrow. The changed ligand–receptor pairs involved antigen presentation, inflammation, apoptosis, and proliferation of B cells. These data showed the transcriptomic landscape of hematopoiesis in AA at single-cell resolution, providing new insights into hematopoiesis failure related with aberrance of B cells, and provide available targets of treatment for AA.

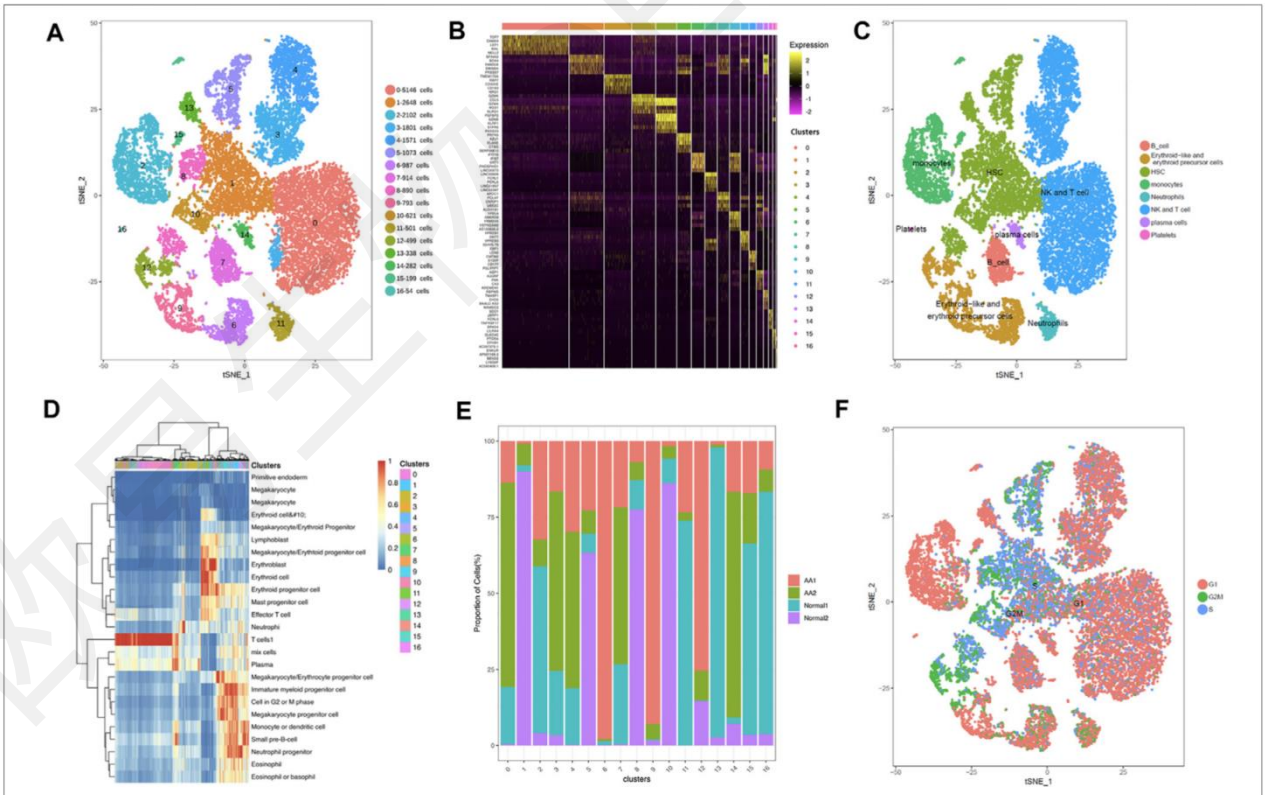

FIGURE 1 | Overview of cell heterogeneity in samples from AA patients and healthy donors. Totally 17 cell clusters were identified by scRNA-seq. **(A)** Totally 17 cell clusters were identified by heat-distributed stochastic neighbor embedding (tSNE). **(B)** Marker genes of each cell cluster. **(C)** Cell type identification and distribution in each cell cluster. **(D)** Heatmap depicting representative differentially expressed genes from each cell cluster. **(E)** Proportions of cell clusters in each sample. **(F)** Cycle of each cell cluster.

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Article

Single-cell RNA-seq and chromatin accessibility profiling decipher the heterogeneity of mouse γδ T cells

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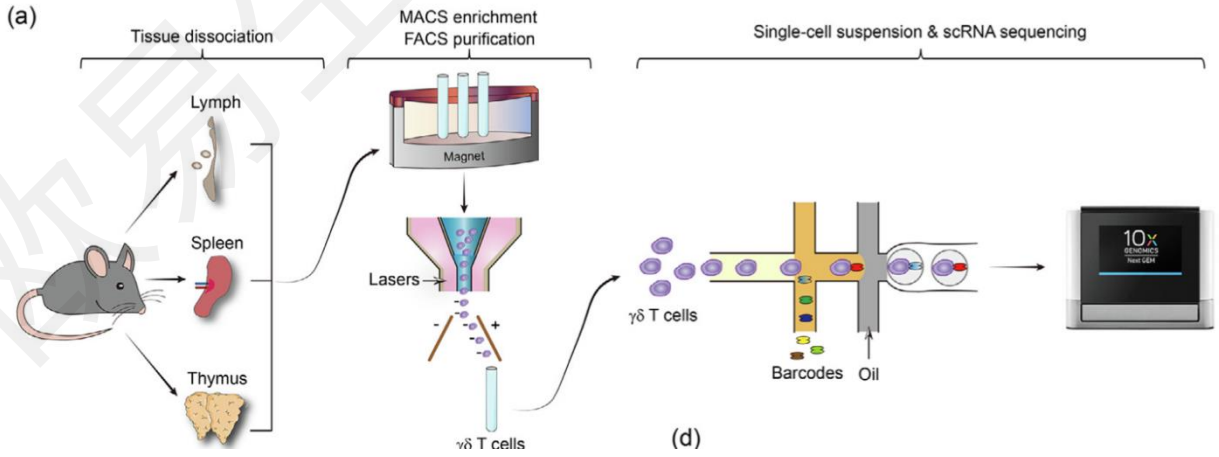
ABSTRACT

The distinct characteristics of cd T cells determine their vital roles in the formation of local immune responses and contribute to tissue homeostasis. However, the heterogeneity of cd T cells across tissues remains unclear. By combining transcriptional and chromatin analyses with a truly unbiased fashion, we constructed a single-cell transcriptome and chromatin accessibility landscape of mouse cd T cells in the lymph, spleen, and thymus. We also revealed the heterogeneity of cd T1 and cd T17 cells across these tissues and inferred their potential regulatory mechanisms. In the thymus, we reconstructed the developmental trajectory and gained further insights into the signature genes from the mature stage, intermediate stage, and immature stage of cd T cells on the basis of single-cell RNA sequencing and single-cell assay for transposase-accessible chromatin sequencing data. Notably, a novel Gzma^{+} cd T cell subset was identified with immature properties and only localized to the thymus. Finally, NR1D1, a circadian transcription factor (TF), was validated as a key and negative regulator of cd T17 cell differentiation by performing a combined analysis of TF motif enrichment, regulon enrichment, and Nr1d1 knockout mice. In summary, our data represent a comprehensive mapping on the transcriptome and chromatin accessibility dynamics of mouse cd T cells, providing a valuable resource and reference for future studies on cd T cells.



KSBMB Korean Society for Biochemistry and Molecular Biology

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ARTICLE OPEN

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Single-cell RNA sequencing reveals B cell–related molecular biomarkers for Alzheimer's disease

Liu-Lin Xiong[1,2,9], Lu-Lu Xue[3,9], Ruo-Lan Du[4,9], Rui-Ze Niu[4], Li Chen[5], Jie Chen[6], Qiao Hu[5], Ya-Xin Tan[4], Hui-Fang Shang[7], Jia Liu[4,9]✉, Chang-Yin Yu[8,9]✉ and Ting-Hua Wang[3,4,5,9]✉

In recent years, biomarkers have been integrated into the diagnostic process and have become increasingly indispensable for obtaining knowledge of the neurodegenerative processes in Alzheimer's disease (AD). Peripheral blood mononuclear cells (PBMCs) in human blood have been reported to participate in a variety of neurodegenerative activities. Here, a single-cell RNA sequencing analysis of PBMCs from 4 AD patients (2 in the early stage, 2 in the late stage) and 2 normal controls was performed to explore the differential cell subpopulations in PBMCs of AD patients. A significant decrease in B cells was detected in the blood of AD patients. Furthermore, we further examined PBMCs from 43 AD patients and 41 normal subjects by fluorescence activated cell sorting (FACS), and combined with correlation analysis, we found that the reduction in B cells was closely correlated with the patients' Clinical Dementia Rating (CDR) scores. To confirm the role of B cells in AD progression, functional experiments were performed in early-stage AD mice in which fibrous plaques were beginning to appear; the results demonstrated that B cell depletion in the early stage of AD markedly accelerated and aggravated cognitive dysfunction and augmented the Aβ burden in AD mice. Importantly, the experiments revealed 18 genes that were specifically upregulated and 7 genes that were specifically downregulated in B cells as the disease progressed, and several of these genes exhibited close correlation with AD. These findings identified possible B cell-based AD severity, which are anticipated to be conducive to the clinical identification of AD progression.

Experimental & Molecular Medicine; https://doi.org/10.1038/s12276-021-00714-8

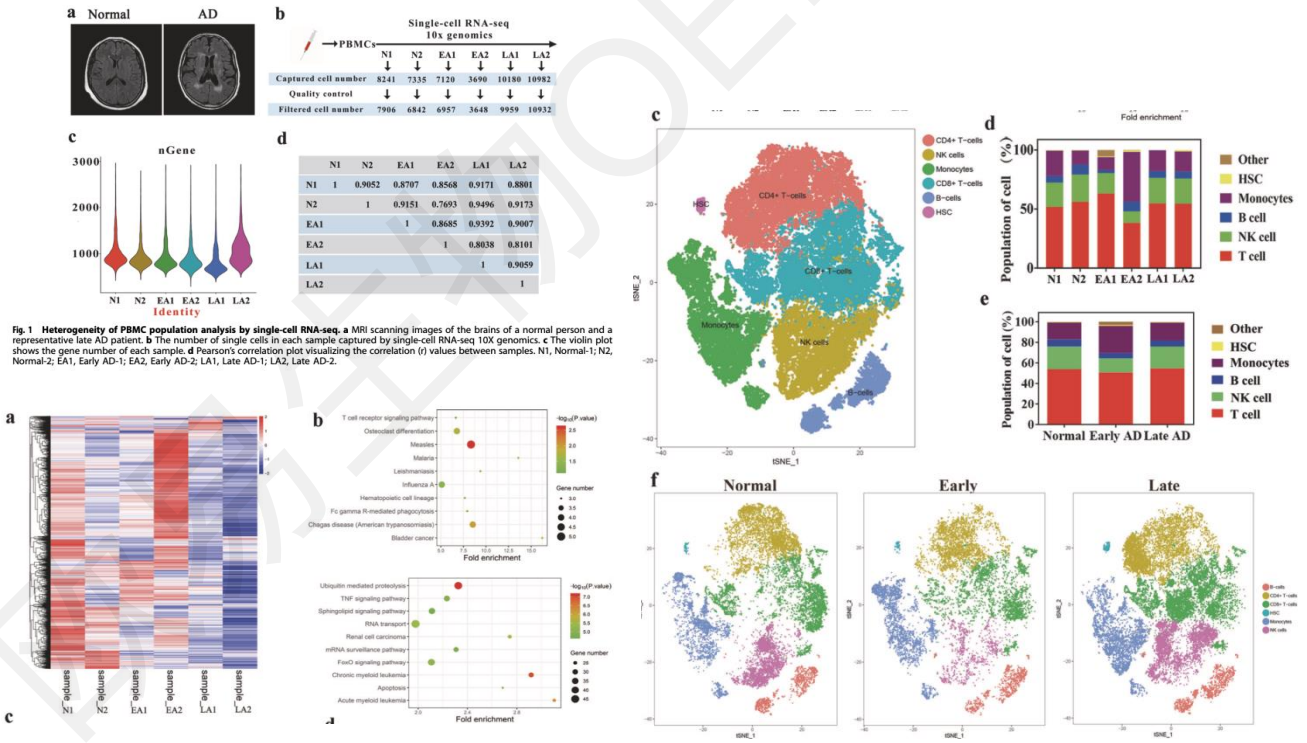

Fig. 1 **Heterogeneity of PBMC population analysis by single-cell RNA-seq. a** MRI scanning images of the brains of a normal person and a representative late AD patient. **b** The number of single cells in each sample captured by single-cell RNA-seq 10X genomics. **c** The violin plot shows the gene number of each sample. **d** Pearson's correlation plot visualizing the correlation (*r*) values between samples. N1, Normal-1; N2, Normal-2; EA1, Early AD-1; EA2, Early AD-2; LA1, Late AD-1; LA2, Late AD-2.

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Cell & Bioscience

RESEARCH Open Access

From nasal to basal: single-cell sequencing of the bursa of Fabricius highlights the IBDV infection mechanism in chickens

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Abstract

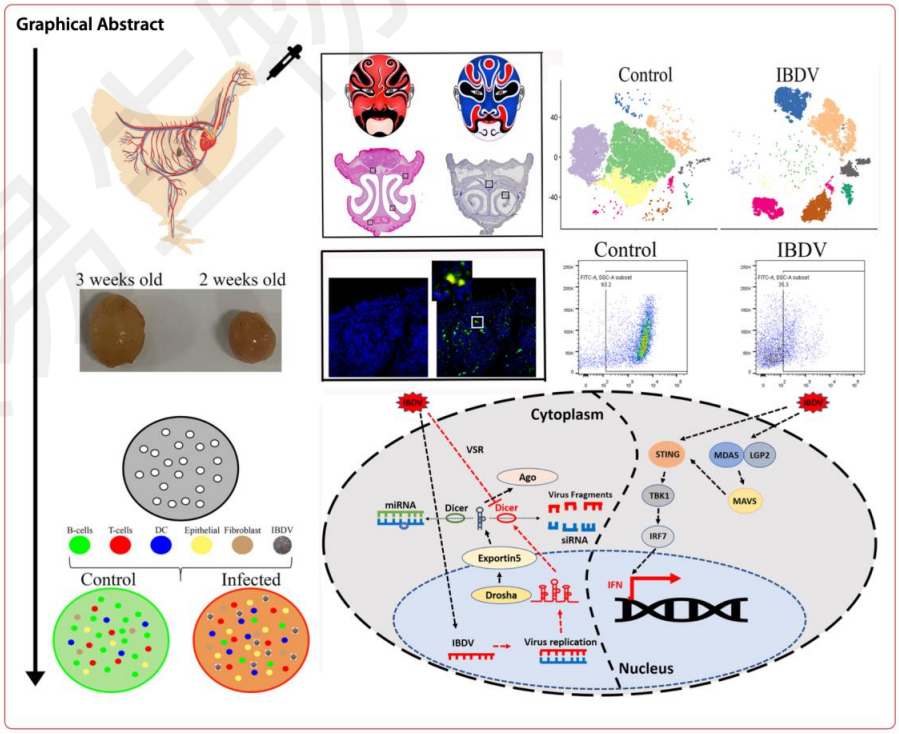
Background: Chickens, important food animals and model organisms, are susceptible to many RNA viruses that invade via the nasal cavity. To determine the nasal entry site of the virus and clarify why avians are susceptible to RNA viruses, infectious bursal disease virus (IBDV) was selected because it is a typical avian RNA virus that infects chickens mainly via the nasal route.

Results: First, we found that IBDV infected the posterior part of the nasal cavity in chickens, which is rich in lymphoid tissue and allows the virus to be easily transferred to the blood. Via the blood circulation, IBDV infected peripheral blood mononuclear cells (PBMCs) and was transferred to the bursa of Fabricius to damage the IgM + B lymphocyte population. Subsequently, the single-cell RNA sequencing (scRNA-seq) results suggested the more detailed response of different bursal cell populations (B cells, epithelial cells, dendritic cells, and fibroblasts) to IBDV. Regarding B cells, IBDV infection greatly decreased the IgM + B cell population but increased the IgA + B cell population in the bursal follicles. In contrast to B cells, bursal epithelial cells, especially basal cells, accumulated a large number of IBDV particles. Furthermore, we found that both innate RNA sensors and interferon-stimulated genes (ISGs) were highly expressed in the IBDV-infected groups, while *dicer* and *ago2* expression was largely blocked by IBDV infection. This result suggests that *dicer*-related RNA interference (RNAi) might be an effective antiviral strategy for IBDV infection in avian.

Conclusion: Our study not only comprehensively elaborates on the transmission of airborne IBDV via the intranasal route and establishes the main target cell types for productive IBDV infection but also provides sufficient evidence to explain the cellular antiviral mechanism against IBDV infection.

Keywords: Nasal cavity, Infectious bursal disease virus, Bursa of Fabricius, Single cell RNA-sequence, RNA interference

Graphical Abstract



Cellular and Molecular Life Sciences _####################_
https://doi.org/10.1007/s00018-021-04044-w
Cellular and Molecular Life Sciences

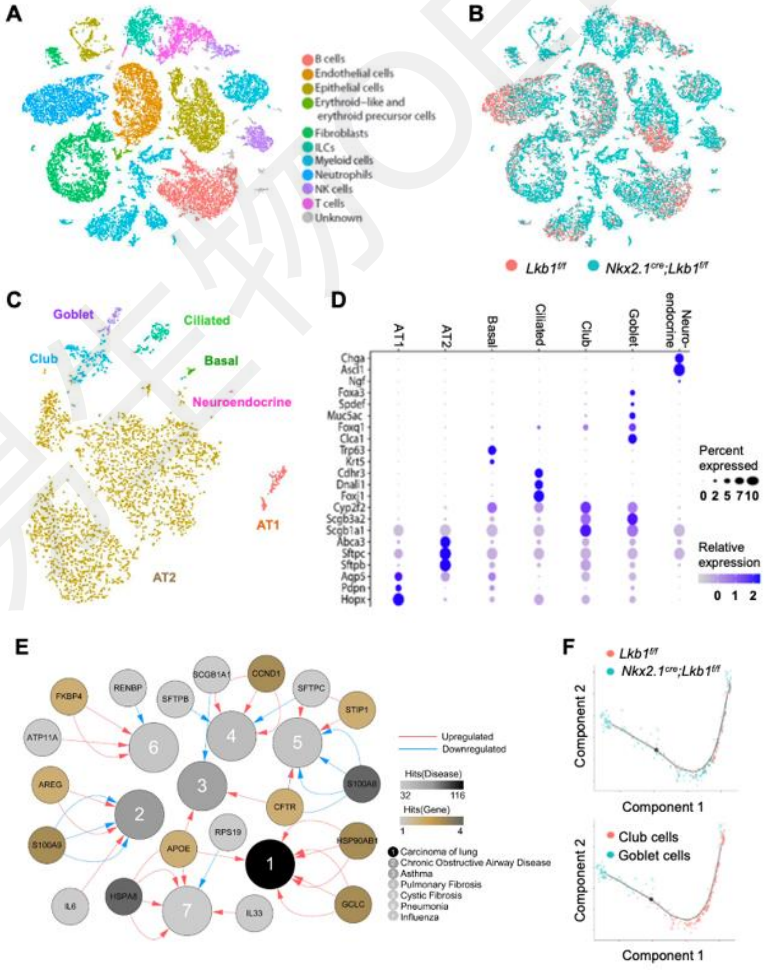
ORIGINAL ARTICLE

LKB1 deficiency upregulates RELM-α to drive airway goblet cell metaplasia

Yu Li[1,2,3,4] · Qiuyang Zhang[1,2,3,4] · Li Li[5] · De Hao[1] · Peiyong Cheng[1] · Kuan Li[1,2,3,4] · Xue Li[1,2,3,4] · Jianhai Wang[1,2,3,4] · Qi Wang[2] · Zhongchao Du[2] · Hongbin Ji[6] · Huaiyong Chen[1,2,3,4]

Abstract
Targeting airway goblet cell metaplasia is a novel strategy that can potentially reduce the chronic obstructive pulmonary disease (COPD) symptoms. Tumor suppressor liver kinase B1 (LKB1) is an important regulator of the proliferation and differentiation of stem/progenitor cells. In this study, we report that LKB1 expression was downregulated in the lungs of patients with COPD and in those of cigarette smoke-exposed mice. *Nkx2.1*^{Cre}*; Lkb1*$^{f/f}$ mice with conditional loss of *Lkb1* in mouse lung epithelium displayed airway mucus hypersecretion and pulmonary macrophage infiltration. Single-cell transcriptomic analysis of the lung tissues from *Nkx2.1*^{Cre}*; Lkb1*$^{f/f}$ mice further revealed that airway goblet cell differentiation was altered in the absence of LKB1. An organoid culture study demonstrated that Lkb1 deficiency in mouse airway (club) progenitor cells promoted the expression of FIZZ1/RELM-α, which drove airway goblet cell differentiation and pulmonary macrophage recruitment. Additionally, monocyte-derived macrophages in the lungs of *Nkx2.1*^{Cre}*; Lkb1*$^{f/f}$ mice exhibited an alternatively activated M2 phenotype, while expressing RELM-α, which subsequently aggravated airway goblet cell metaplasia. Our findings suggest that the LKB1-mediated crosstalk between airway progenitor cells and macrophages regulates airway goblet cell metaplasia. Moreover, our data suggest that LKB1 agonists might serve as a potential therapeutic option to treat respiratory disorders associated with goblet cell metaplasia.

Keywords Cystic fibrosis · Asthma · Lung stem and progenitor cells · Crosstalk · Cell fate · Polarization



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RESEARCH ARTICLE

CLINICAL AND TRANSLATIONAL MEDICINE Open Access WILEY

Single-cell transcriptome atlas of human mesenchymal stem cells exploring cellular heterogeneity

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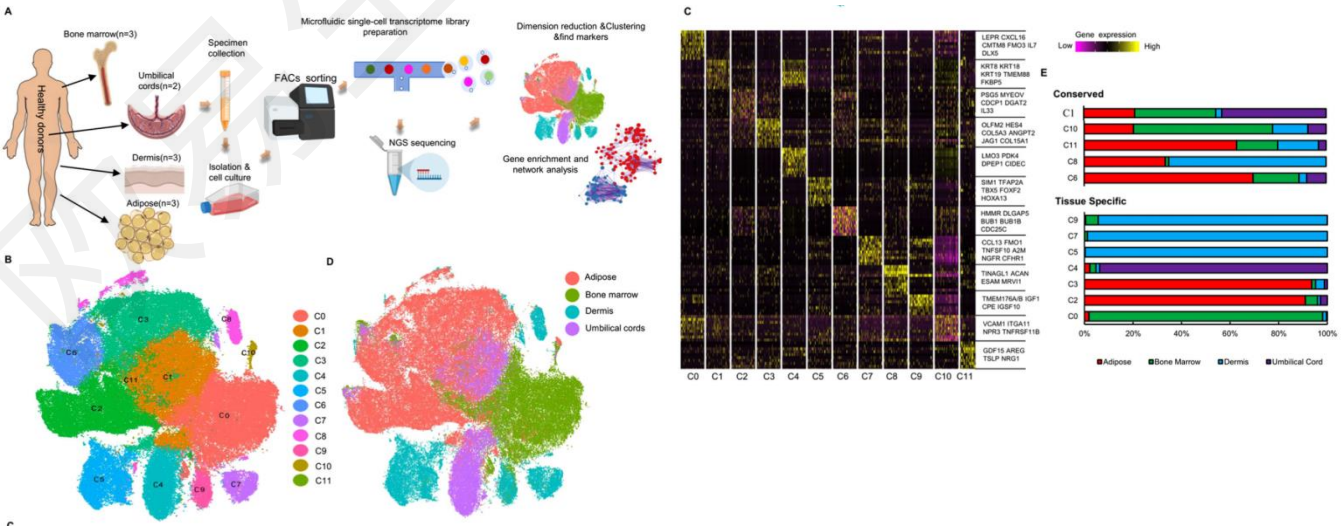
Abstract

Background: The heterogeneity of mesenchymal stem cells (MSCs) is poorly understood, thus limiting clinical application and basic research reproducibility. Advanced single-cell RNA sequencing (scRNA-seq) is a robust tool used to anal- yse for dissecting cellular heterogeneity. However, the comprehensive single-cell atlas for human MSCs has not been achieved.

Methods: This study used massive parallel multiplexing scRNA-seq to construct an atlas of > 130 000 single-MSC transcriptomes across multiple tissues and donors to assess their heterogeneity. The most widely clinically utilised tissue resources for MSCs were collected, including normal bone marrow (n = 3), adi- pose (n = 3), umbilical cord (n = 2), and dermis (n = 3).

Results: Seven tissue-specific and five conserved MSC subpopulations with distinct gene-expression signatures were identified from multiple tissue ori- gins based on the high-quality data, which has not been achieved previously. This study showed that extracellular matrix (ECM) highly contributes to MSC heterogeneity. Notably, tissue-specific MSC subpopulations were sub- stantially heterogeneous on ECM-associated immune regulation, antigen processing/presentation, and senescence, thus promoting inter-donor and intra-tissue heterogeneity. The variable dynamics of ECM-associated genes had discrete trajectory patterns across multiple tissues. Additionally, the conserved and tissue-specific transcriptomic-regulons and protein-protein interactions were identified, potentially representing common or tissue-specific MSC func- tional roles. Furthermore, the umbilical-cord-specific subpopulation possessed advantages in immunosuppressive properties.



Systematic search for schizophrenia pathways sensitive to perturbation by immune activation

Yue Gao[1,2,3], Xiaozhen Liang[1], Zhonglu Ren[1], Yanjun Li[1,2,3] and Xinping Yang[1,2,3*]

Abstract

Immune activation has been recently found to play a large part in the development of schizophrenia, but the underlying mechanism remains largely unknown. Here, we report the construction of a high-quality protein interaction network for schizophrenia (SCZ Network) using a "neighborhood walking" approach to searching across human interactome network for disease-related neighborhoods. The spatiotemporal expression pattern of the immune genes in the SCZ Network demonstrates that this disease network is sensitive to the perturbation of immune activation during mid- to late fetal development and adolescence. The immune genes in the network are involved in pathways regulating the formation, structure and function of synapses and neural connections. Using single-cell transcriptome sequencing on the brains of immune-activated mice, we found that immune activation disturbed the SCZ network in the major brain cell types and the dysregulated pathways were also involved in synapse regulation, demonstrating that our "neighborhood walking" approach enables biological discovery in complex disorders.

Key words: Complex disease, Schizophrenia, disease candidate genes, immune genes, protein-protein interaction network, neighborhood walking, disease network, synapse remodeling

Low *XIST* expression in Sertoli cells of Klinefelter syndrome patients caused the high susceptibility of these cells to an extra X chromosome

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Abstract：

Klinefelter syndrome (KS) is the most common genetic cause of human male infertility. Patients suffer from heterogeneous testicular atrophy with loss of both germ cells and Sertoli cells. However, the mechanism by which the extra X chromosome causes failure of spermatogenesis remains poorly understood. Here, we profiled testicular single-cell transcriptomes from three KS patients and compared the results with those of healthy donors. Among different somatic cells, Sertoli cells showed the greatest changes in KS patients. Further analysis showed that XIST, a key long intergenic non-coding RNA that inactivates one X chromosome in female mammals, was widely expressed in somatic cells, except for Sertoli cells, leading to an increase in X-inactivation genes in these cells, which may cause Sertoli cells death and disruption of the spermatogenic provided a theoretical basis for subsequent research and related treatment.

Key words：Single-cell genomics; Klinefelter syndrome; spermatogenesis; non-obstructive azoospermia; Sertoli cell; X inactivation

Dihydroartemisinin Shows Promising Effects in the Treatment of Experimental Autoimmune Encephalomyelitis and Maintains Inflammatory Homeostasis by Targeting AXL in Microglia

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Abstract

Background: During EAE progression, the endogenous mechanisms mediating nervous autoimmune inflammation balance, as represented by AXL, were proved to be pathologically disturbed, immune balance and axon repair. Therapeutically, by activating AXL signaling, the inflammatory rebalance from promotion to resolution has attracted increasing attention and showed advantages in autoimmune disease treatment. Previous studies implied that DHA had potential effects in treating autoimmune diseases. However, the detailed mechanisms in inflammation regulation, especially in CNS, remain unclear.

Methods: C57BL/6 mice were immunized with MOG_{35-55} and treated daily with DHA. Then clinical scores, pathology, and ethology features of EAE were assessed through histological staining (H&E, LFB staining), TEM and gait analysis. Moreover, DHA-responsive cells and genes were screened by 10x Genomics. The immunological responses to DHA were measured by flow cytometry and fluorescence microscope in BV2 cells. The concentrations and bio-activities of chemokines were respectively evaluated through ELISA and trans-well assay.

Results: After DHA treatment, the clinical scores and body weight were significantly improved. Histologically, mice showed slighter spinal cord lesion, less inflammatory cuffs. By using gait analysis, DHA obviously improved physical coordination. 10x Genomics demonstrated that DHA selectively upregulated AXL expression in microglia. Immunologically, by enhancing AXL signaling, the phagocytic and chemotactic potential of microglia and the Treg differentiation followed by upregulating PDL1 were significantly influenced by DHA. Conversely, specific blocking of AXL by SGI7079 was sufficient to reverse above-mentioned functions. Molecularly, DHA specifically rebalanced the overactivated inflammation through STAT1:SOCS3: AXL: IFNAR pathway.

Conclusions: The present study highlighted the central role of AXL signaling in DHA mediated inflammatory transition.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
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Single-Cell RNA Sequencing of the Rat Carotid Arteries Uncovers Potential Cellular Targets of Neointimal Hyperplasia

Xiao-Fei Gao [1,2†], Ai-Qun Chen [1†], Zhi-Mei Wang [1†], Feng Wang [1], Shuai Luo [1], Si-Yu Chen [1], Yue Gu [1], Xiang-Quan Kong [1], Guang-Feng Zuo [1], Yan Chen [3], Zhen Ge [1], Jun-Jie Zhang [1,2] and Shao-Liang Chen [1,2]*

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Aims: In-stent restenosis (ISR) remains an Achilles heel of drug-eluting stents despite technical advances in devices and procedural techniques. Neointimal hyperplasia (NIH) is the most important pathophysiological process of ISR. The present study mapped normal arteries and stenotic arteries to uncover potential cellular targets of neointimal hyperplasia.

Methods and Results: By comparing the left (control) and right (balloon injury) carotid arteries of rats, we mapped 11 clusters in normal arteries and 11 mutual clusters in both the control and experimental groups. Different clusters were categorized into 6 cell types, including vascular smooth muscle cells (VSMCs), fibroblasts, endothelial cells (ECs), macrophages, unknown cells and others. An abnormal cell type expressing both VSMC and fibroblast markers at the same time was termed a transitional cell *via* pseudotime analysis.

Due to the high proportion of VSMCs, we divided them into 6 clusters and analyzed their relationship with VSMC phenotype switching. Moreover, N-myristoyltransferase 1 (NMT1) was verified as a credible VSMC synthetic phenotype marker. Finally, we proposed several novel target genes by disease susceptibility gene analysis, such as Cyp7a1 and Cdk4, which should be validated in future studies.

Conclusion: Maps of the heterogeneous cellular landscape in the carotid artery were defined by single-cell RNA sequencing and revealed several cell types with their internal relations in the ISR model. This study highlights the crucial role of VSMC phenotype switching in the progression of neointimal hyperplasia and provides clues regarding the underlying mechanism of NIH.

Keywords: in-stent restenosis, single-cell sequencing, vascular smooth muscle cell, transitional-cell, neointimal hyperplasia

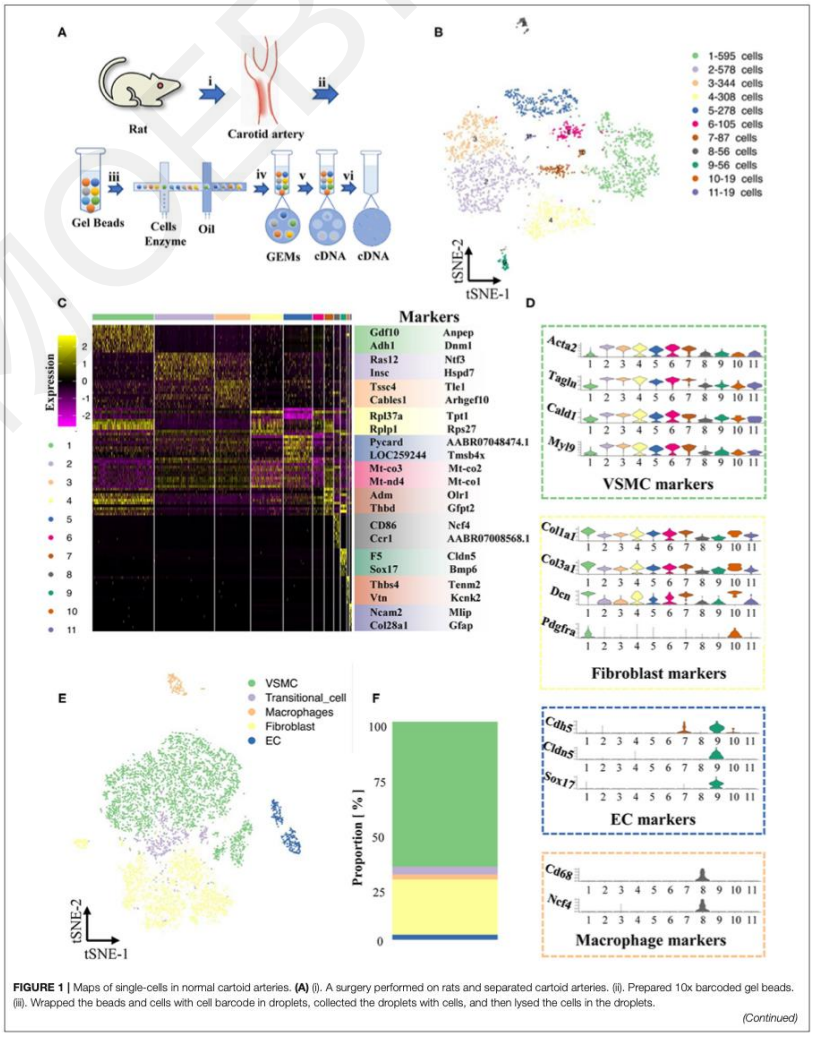

FIGURE 1 | Maps of single-cells in normal cartoid arteries. **(A)** (i). A surgery performed on rats and separated cartoid arteries. (ii). Prepared 10x barcoded gel beads. (iii). Wrapped the beads and cells with cell barcode in droplets, collected the droplets with cells, and then lysed the cells in the droplets.

(Continued)

RESEARCH ARTICLE

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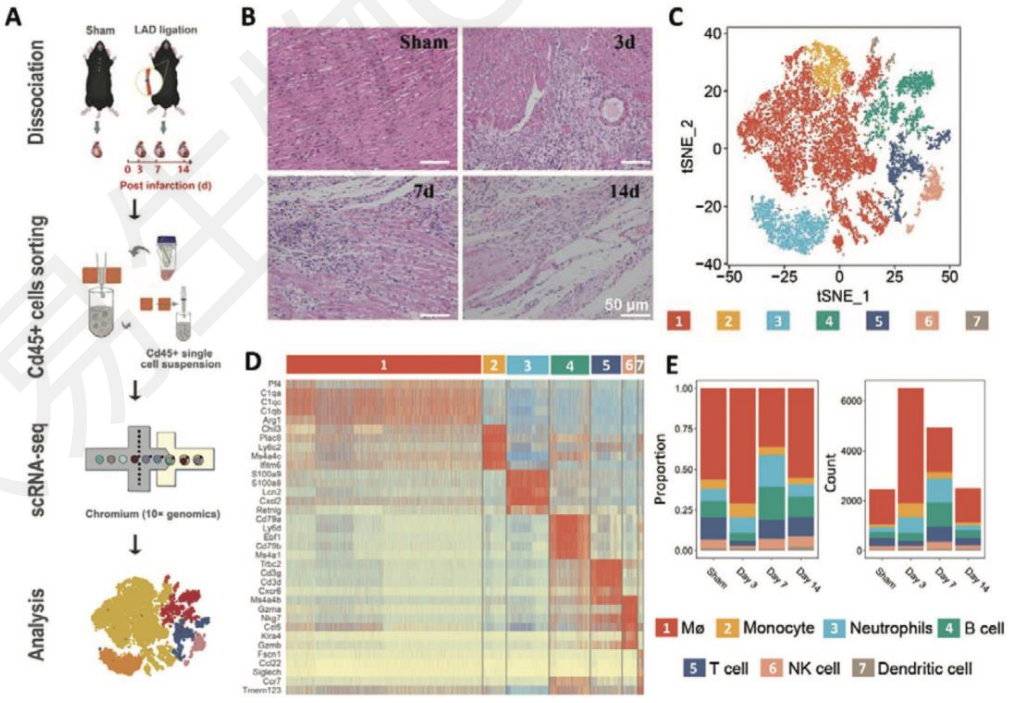
Single-Cell RNA Sequencing Reveals the Temporal Diversity and Dynamics of Cardiac Immunity after Myocardial Infarction

Kaiyu Jin, Shan Gao, Penghui Yang, Rongfang Guo, Dan Li, Yunsha Zhang, Xiaoyan Lu, Guanwei Fan,* and Xiaohui Fan**

Myocardial infarction (MI) is strongly associated with the temporal regulation of cardiac immunity. However, a variety of current clinical trials have failed because of the lack of post-MI immunomodulating/anti-inflammatory targets. Single-cell RNA sequencing analysis of the cardiac *Cd45*$^{+}$ immune cell at 0, 3, 7, and 14 d after injury in a mouse left anterior descending coronary artery ligation model is performed. Major immune cell populations, distinct subsets, and dynamic changes are identified. Macrophages (Mø) are most abundant, peaking at 3 d after infarction. Mø-5 and Mø-6 are the predominant infiltrated subsets at this time point, with strong expression of inflammatory factors. Further analysis demonstrates that suppressing these sets attenuated pathological MI progression by preventing subsequent leukocyte extravasation and adverse remodeling. Abundant apoptotic neutrophils and a profibrotic macrophage subset on days 7 and 14, respectively, are also detected. These results provide a basis for developing cell type- and time-specific interventions in MI.



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Particle and Fibre Toxicology

RESEARCH Open Access

Coal dust exposure triggers heterogeneity of transcriptional profiles in mouse pneumoconiosis and Vitamin D remedies

Min Mu[1,2,3,4†], Bing Li[3†], Yuanjie Zou[3], Wenyang Wang[3], Hangbing Cao[3], Yajun Zhang[1,2,3,4], Qixian Sun[3], Haoming Chen[3], Deyong Ge[1,2,3,4], Huihui Tao[1,2,3,4], Dong Hu[1,2,3,4], Liang Yuan[1,2,3,4*], Xinrong Tao[1,2,3,4*] and Jianhua Wang[1,5*]

Abstract

Background: Coal dust particles (CDP), an inevitable by-product of coal mining for the environment, mainly causes coal workers' pneumoconiosis (CWP). Long-term exposure to coal dust leads to a complex alternation of biological processes during regeneration and repair in the healing lung. However, the cellular and complete molecular changes associated with pulmonary homeostasis caused by respiratory coal dust particles remain unclear.

Methods: This study mainly investigated the pulmonary toxicity of respirable-sized CDP in mice using unbiased single-cell RNA sequencing. CDP (< 5 μm) collected from the coal mine was analyzed by Scanning Electron Microscope (SEM) and Mass Spectrometer. In addition, western blotting, Elisa, QPCR was used to detect gene expression at mRNA or protein levels. Pathological analysis including HE staining, Masson staining, immunohistochemistry, and immunofluorescence staining were performed to characterize the structure and functional alternation in the pneumoconiosis mouse and verify the reliability of single-cell sequencing results.

Results: SEM image and Mass Spectrometer analysis showed that coal dust particles generated during coal mine production have been crushed and screened with a diameter of less than 5 μm and contained less than 10% silica. Alveolar structure and pulmonary microenvironment were destroyed, inflammatory and death (apoptosis, autophagy, and necrosis) pathways were activated, leading to pneumoconiosis in post 9 months coal dust stimulation. A distinct abnormally increased alveolar type 2 epithelial cell (AT2) were classified with a highly active state but reduced the antimicrobial-related protein expression of LYZ and Chia1 after CDP exposure. Beclin1, LC3B, LAMP2, TGF-ß, and MLPH were up-regulated induced by CDP, promoting autophagy and pulmonary fibrosis. A new subset of macrophages with M2-type polarization double expressed MLPH + /CD206 + was found in mice having pneumoconiosis but markedly decreased after the Vitamin D treatment. Activated MLPH + /CD206 + M2 macrophages secreted TGF-β1 and are sensitive to Vitamin D treatment.

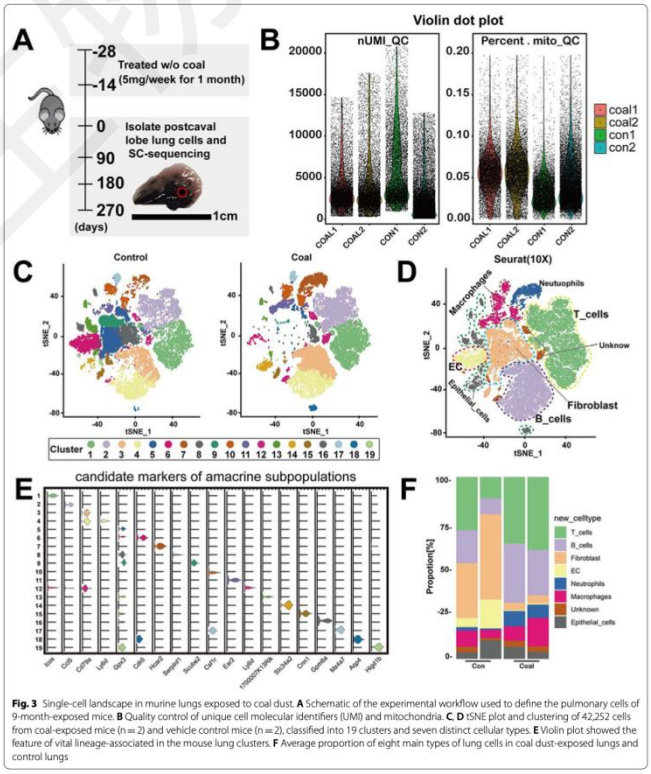

Fig. 3 Single-cell landscape in murine lungs exposed to coal dust. **A** Schematic of the experimental workflow used to define the pulmonary cells of 9-month-exposed mice. **B** Quality control of unique cell molecular identifiers (UMI) and mitochondria. **C**, **D** tSNE plot and clustering of 42,252 cells from coal-exposed mice (n = 2) and vehicle control mice (n = 2), classified into 19 clusters and seven distinct cellular types. **E** Violin plot showed the feature of vital lineage-associated in the mouse lung clusters. **F** Average proportion of eight main types of lung cells in coal dust-exposed lungs and control lungs

Molecular Therapy
Nucleic Acids
Original Article

Heterogeneity of human corneal endothelium implicates lncRNA *NEAT1* in Fuchs endothelial corneal dystrophy

Qun Wang,[1,2,5] Shengqian Dou,[1,2,5] Bin Zhang,[1,2] Hui Jiang,[1,2] Xia Qi,[1,2] Haoyun Duan,[1,2] Xin Wang,[1,4] Chunxiao Dong,[1,4] Bi Ning Zhang,[1,2] Lixin Xie,[1,2] Yihai Cao,[3] Qingjun Zhou,[1,2] and Weiyun Shi[1,4]

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The corneal endothelium is critical for maintaining corneal clarity by mediating hydration through barrier and pump func- tions. Progressive loss of corneal endothelial cells during aging has been associated with the development of Fuchs endothelial corneal dystrophy (FECD), one of the main causes of cornea- related vision loss. The mechanisms underlying FECD develop- ment remain elusive. Single-cell RNA sequencing of isolated healthy human corneas discovered 4 subpopulations of corneal endothelial cells with distinctive signatures. Unsupervised clus- tering analysis uncovered nuclear enriched abundant tran- script 1 (NEAT1), a long non-coding RNA (lncRNA), as the top expressed gene in the C0-endothelial subpopulation, but markedly downregulated in FECD. Consistent with human corneas, a UVA-induced mouse FECD model validated the loss of NEAT1 expression. Loss of NEAT1 function by an in vivo genetic approach reproduced the exacerbated phenotype of FECD by ablating corneal endothelial cells. Conversely, gain of function by a CRISPR-activated adenoviral delivery system protected corneas from UVA-induced FECD. Our findings provide novel mechanistic insights into the development of FECD, and targeting NEAT1 offers an attractive approach for treating FECD.

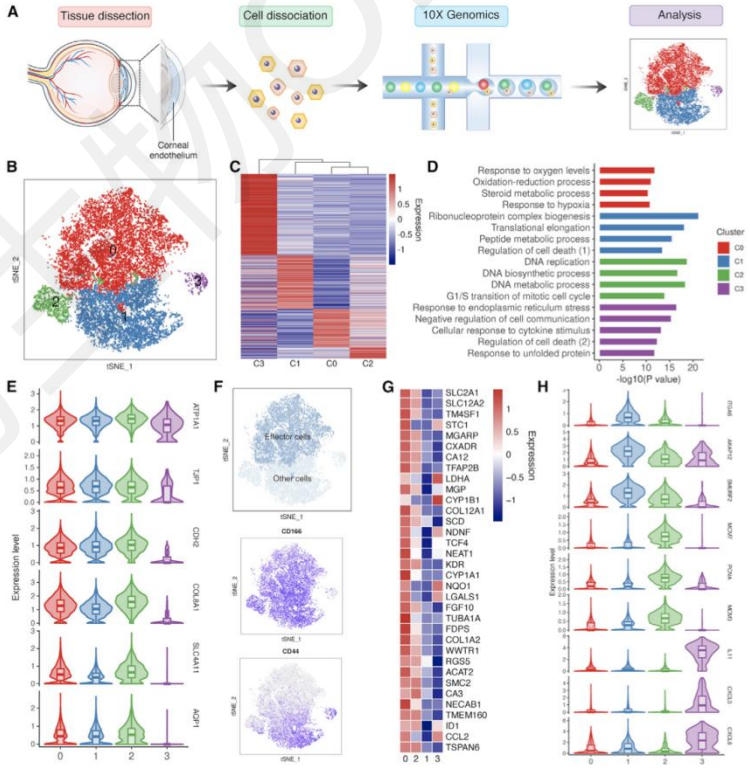

Figure 1. Construction of human corneal endothelium atlas by scRNA-seq

(A) Flowchart of the experiments performed in this study. (B) t-SNE clustering of human corneal endothelial cells colored by 4 distinct clusters. (C) Heatmap for expression of differentially expressed genes (DEGs) in each subtype. (D) Representative GO terms of all DEGs in each cell type. (E) Expression of representative marker genes across clusters. (F) Definition and surface phenotype of "effector cells." (G) Expression of top markers in "effector cells." (H) Representative novel markers of C1–C3 cells.

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Cell & Bioscience

RESEARCH Open Access

Transcriptome-scale spatial gene expression in rat arcuate nucleus during puberty

Shasha Zhou[1,2†], Shaolian Zang[2†], Yanping Hu[3†], Yifen Shen[1†], Hua Li[4], Wenlian Chen[5], Pin Li[2*] and Yihang Shen[1*]

Abstract

Background: A variety of neurons in hypothalamus undergo a complicated regulation on transcription activity of multiple genes for hypothalamic–pituitary–gonadal axis activation during pubertal development. Identification of puberty-associated cell composition and characterization of the unique transcriptional signatures across different cells are beneficial to isolation of specific neurons and advanced understanding of their functions.

Methods: The hypothalamus of female Sprague–Dawley rats in postnatal day-25, 35 and 45 were used to define the dynamic spatial atlas of gene expression in the arcuate nucleus (ARC) by 10× Genomics Visium platform. A surface protein expressed selectively by kisspeptin neurons was used to sort neurons by flow cytometric assay in vitro. The transcriptome of the isolated cells was examined using Smart sequencing.

Results: Four subclusters of neurons with similar gene expression signatures in ARC were identified. Only one subcluster showed the robust expression of Kiss1, which could be isolated by a unique membrane surface biomarker Solute carrier family 18 member A3 (SLC18A3). Moreover, genes in different subclusters presenting three expression modules distinctly functioned in each pubertal stage. Different types of cells representing distinct functions on glial or neuron differentiation, hormone secretion as well as estradiol response precisely affect and coordinate with each other, resulting in a complicated regulatory network for hypothalamic–pituitary–gonadal axis initiation and modulation.

Conclusion: Our data revealed a comprehensive transcriptomic overview of ARC within different pubertal stages, which could serve as a valuable resource for the study of puberty and sexual development disorders.

Keywords: Puberty, Hypothalamus, Spatial transcriptome, ARC, Kiss1, Slc18a3

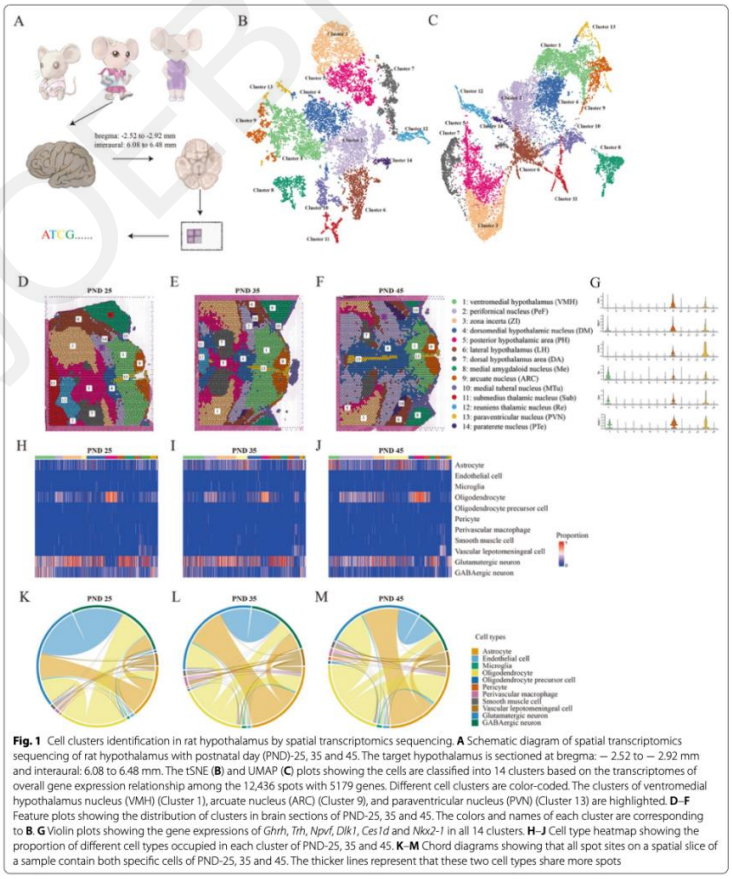

Fig. 1 Cell clusters identification in rat hypothalamus by spatial transcriptomics sequencing. **A** Schematic diagram of spatial transcriptomics sequencing of rat hypothalamus with postnatal day (PND)-25, 35 and 45. The target hypothalamus is sectioned at bregma: − 2.52 to − 2.92 mm and interaural: 6.08 to 6.48 mm. The tSNE (**B**) and UMAP (**C**) plots showing the cells are classified into 14 clusters based on the transcriptomes of overall gene expression relationship among the 12,436 spots with 5179 genes. Different cell clusters are color-coded. The clusters of ventromedial hypothalamus nucleus (VMH) (Cluster 1), arcuate nucleus (ARC) (Cluster 9), and paraventricular nucleus (PVN) (Cluster 13) are highlighted. **D**–**F** Feature plots showing the distribution of clusters in brain sections of PND-25, 35 and 45. The colors and names of each cluster are corresponding to **B**. **G** Violin plots showing the gene expressions of *Ghrh*, *Trh*, *Npvf*, *Dlk1*, *Ces1d* and *Nkx2-1* in all 14 clusters. **H**–**J** Cell type heatmap showing the proportion of different cell types occupied in each cluster of PND-25, 35 and 45. **K**–**M** Chord diagrams showing that all spot sites on a spatial slice of a sample contain both specific cells of PND-25, 35 and 45. The thicker lines represent that these two cell types share more spots

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ORIGINAL RESEARCH

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Dissecting the heterogeneity of the microenvironment in primary and recurrent nasopharyngeal carcinomas using single-cell RNA sequencing

Wen-Sa Peng[a,b], Xin Zhou[a,b], Wen-Bin Yan[a,b], Yu-Jiao Li[a,b], Cheng-Run Du[a,b], Xiao-Shen Wang[c], Chun-Ying Shen[a,b], Qi-Feng Wang[b,d], Hong-Mei Ying[a,b], Xue-Guan Lu[a,b], Ting-Ting Xu[a,b], and Chao-Su Hu[a,b]

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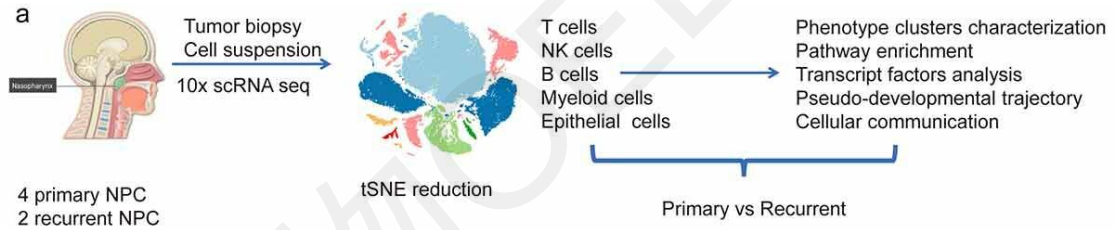
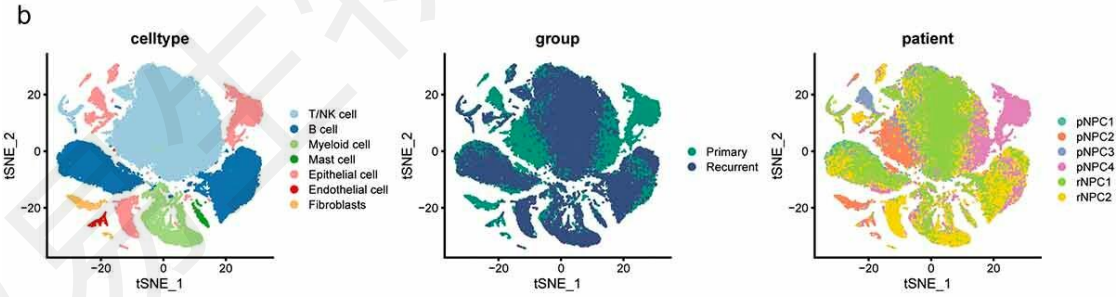
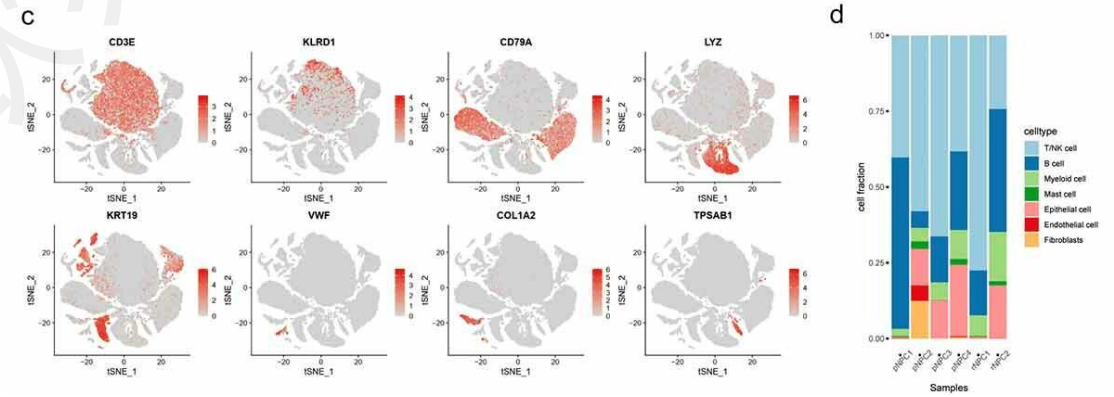
ABSTRACT

Nasopharyngeal carcinoma (NPC) has a 10–15% recurrence rate, while no long term or durable treatment options are currently available. Single-cell profiling in recurrent NPC (rNPC) may aid in designing effective anticancer therapies, including immunotherapies. For the first time, we profiled the transcriptomes of ~60,000 cells from four primary NPC and two rNPC cases to provide deeper insights into the dynamic changes in rNPC within radiation fields. Heterogeneity of both immune cells (T, natural killer, B, and myeloid cells) and tumor cells was characterized. Recurrent samples showed increased infiltration of regulatory T cells in a highly immunosuppressive state and $CD8^+$ T cells in a highly cytotoxic and dysfunctional state. Enrichment of M2-polarized macrophages and $LAMP3^+$ dendritic cells conferred enhanced immune suppression to rNPC. Furthermore, malignant cells showed enhanced immune-related features, such as antigen presentation. Elevated regulatory T cell levels were associated with a worse prognosis, with certain receptor-ligand communication pairs identified in rNPC. Even with relatively limited samples, our study provides important clues to complement the exploitation of rNPC immune environment and will help advance targeted immunotherapy of rNPC.







Identification of Novel Regulators Required for Early Development of Vein Pattern in the Cotyledons by Single-cell RNA-seq

Zhixin Liu[1#], Jiajing Wang[1#], Yaping Zhou[1], Yixin Zhang[1], Aizhi Qin[1], Xiaole Yu[1], Zihao Zhao[1], Rui Wu[1], Chenxi Guo[1], George Bawa[1], Jean-David Rochaix[2], Xuwu Sun [1*]

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Running Title: Single-Cell RNA-Seq of Leaf Vein

Keywords: Novel regulators, Development, Leaf veins, Cotyledons, Single-cell RNA-seq, *Arabidopsis thaliana*

SUMMARY

The leaf veins of higher plants contain a highly specialized vascular system comprised of xylem and phloem cells which transport water, organic compounds and mineral nutrients. The development of the vascular system is controlled by phytohormones which interact with complex transcriptional regulatory networks. Before the emergence of true leaves, the cotyledons of young seedlings perform photosynthesis which provides energy for the sustainable growth and survival of seedlings. However, the mechanisms underlying the early development of leaf veins in cotyledons are still not fully understood, in part due to the complex cellular composition of this tissue. To better understand the development of leaf veins, we analyzed 14,117 single cells from 3-day-old cotyledons using single cell RNA sequencing. Based on gene expression patterns, we identified 10 clusters of cells and traced their developmental trajectories. We discovered multiple new marker genes and developmental features of leaf veins. The transcription factor networks of some cell types indicated potential roles of CYCLING DOF FACTOR 5 (CDF5) and REPRESSOR OF GA (RGA) in the early development and function of the leaf veins in cotyledons. These new findings lay a foundation for understanding the early developmental dynamics of cotyledon veins.

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The Crop Journal

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Single-cell RNA sequencing reveals the landscape of maize root tips and assists in identification of cell type-specific nitrate-response genes

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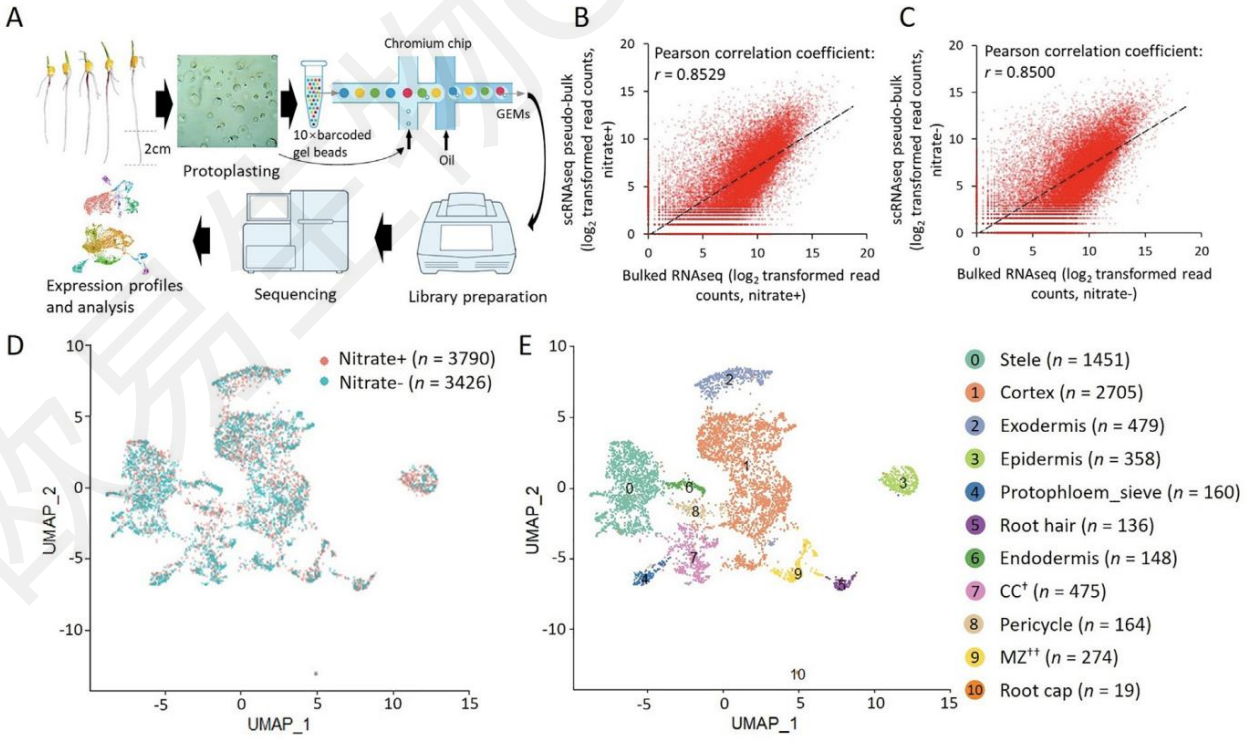
ABSTRACT

The root system is fundamental for maize growth and yield. Characterizing its heterogeneity and cell type-specific response to nitrate at the single-cell level will shed light on root development and nutrient uptake. We profiled the transcriptomes of >7000 cells derived from root tips of maize seedlings grown on media with or without nitrate, and identified 11 major cell types or tissues and 85 cell type-specific nitrate-response genes, including several known nitrate metabolic genes. A pseudotime analysis showed a continuous pseudotime series with the beginning at meristematic zone cells and showed that the root hair cell was derived by differentiation of a subset of epidermal cells. Interspecies comparison of root cells between maize and rice revealed the conservation and divergence of the root cell types and identified 57, 216, and 80 conserved orthologous genes in root hair, endodermis, and phloem cells respectively. This study provides a global view of maize root tip developmental processes and responses to nitrate at the single-cell level. The genes described in the present work could serve as targets for further genetic analyses and accurate regulation of gene expression and phenotypic variation in specific cell types or tissues.



International Journal of *Molecular Sciences*

MDPI

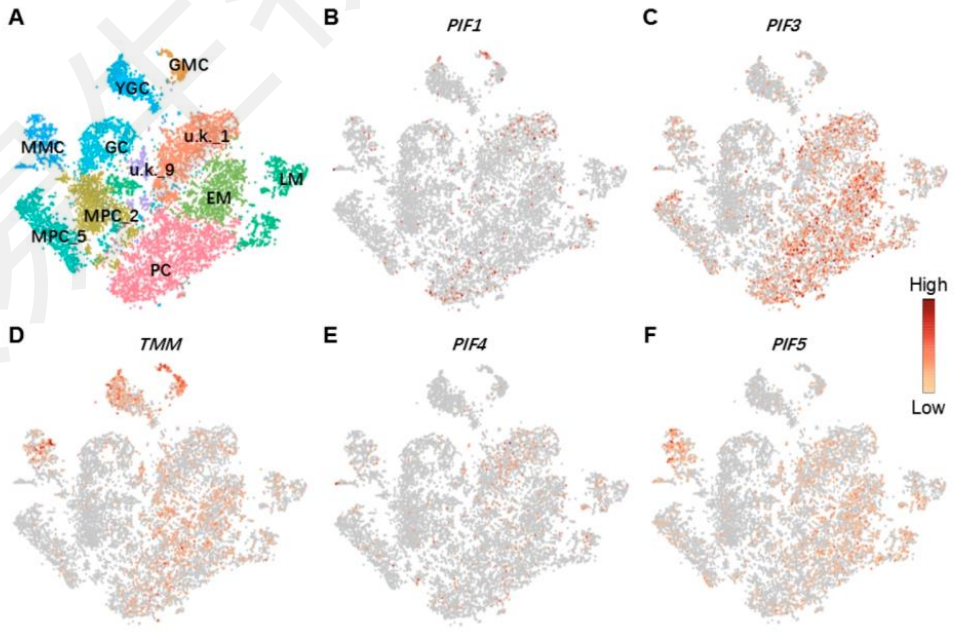
Article

Identification of the Regulators of Epidermis Development under Drought- and Salt-Stressed Conditions by Single-Cell RNA-Seq

Zhixin Liu [1,2,3,†], Chenxi Guo [1,2,3,†], Rui Wu [1,2,3], Jiajing Wang [1,2,3], Yaping Zhou [1,2,3], Xiaole Yu [1,2,3], Yixin Zhang [1,2,3], Zihao Zhao [1,2,3], Hao Liu [1,2,3], Susu Sun [1,2,3], Mengke Hu [1,2,3], Aizhi Qin [1,2,3], Yumeng Liu [1,2,3], Jincheng Yang [1,2,3], George Bawa [1,2,3] and Xuwu Sun [1,2,3,*]

Abstract: As sessile organisms, plants constantly face challenges from the external environment. In order to meet these challenges and survive, plants have evolved a set of sophisticated adaptation strategies, including changes in leaf morphology and epidermal cell development. These developmental patterns are regulated by both light and hormonal signaling pathways. However, our mechanistic understanding of the role of these signaling pathways in regulating plant response to environmental stress is still very limited. By applying single-cell RNA-Seq, we determined the expression pattern of *PHYTOCHROME INTERACTING FACTOR* (*PIF*) *1*, *PIF3*, *PIF4*, and *PIF5* genes in leaf epidermal pavement cells (PCs) and guard cells (GCs). PCs and GCs are very sensitive to environmental stress, and our previous research suggests that these PIFs may be involved in regulating the development of PCs, GCs, and leaf morphology under environmental stress. Growth analysis showed that *pif1/3/4/5* quadruple mutant maintained tolerance to drought and salt stress, and the length to width ratio of leaves and petiole length under normal growth conditions were similar to those of wild-type (WT) plants under drought and salt treatment. Analysis of the developmental patterns of PCs and GCs, and whole leaf morphology, further confirmed that these PIFs may be involved in mediating the development of epidermal cells under drought and salt stress, likely by regulating the expression of *MUTE* and *TOO MANY MOUTHS* (*TMM*) genes. These results provide new insights into the molecular mechanism of plant adaptation to adverse growth environments.

Keywords: PIFs; leaf; epidermal cell; development; drought; salt; scRNA-Seq



frontiers in Cell and Developmental Biology
ORIGINAL RESEARCH
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Single-Cell RNA-Seq Analysis Reveals Macrophage Involved in the Progression of Human Intervertebral Disc Degeneration

Zemin Ling[1†], Yong Liu[2†], Zhe Wang[3†], Ziji Zhang[2], Bolin Chen[1], Jiaming Yang[4], Baozhu Zeng[4], Yu Gao[4], Chang Jiang[3], Yulin Huang[4], Xuenong Zou[1], Xiuhui Wang[5] and Fuxin Wei[4]**

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Intervertebral disc degeneration (IDD) has been considered as the primary pathological mechanism that underlies low back pain. Understanding the molecular mechanisms underlying human IDD is imperative for making strategies to treat IDD-related diseases. Herein, we report the molecular programs, lineage progression patterns, and paths of cellular communications during the progression of IDD using single-cell RNA sequencing (scRNA-seq) on nucleus pulposus (NP) cells from patients with different grades of IDD undergoing discectomy. New subtypes of cells and cell-type-specific gene signatures of the metabolic homeostatic NP cells (Met NPC), adhesive NP cells (Adh NPC), inflammatory response NP cells (IR NPC), endoplasmic reticulum stress NP cells (ERS NPC), fibrocartilaginous NP cells (Fc NPC), and CD70 and $CD82^{+}$ progenitor NP cells (Pro NPC) were identified. In the late stage of IDD, the IR NPC and Fc NPC account for a large proportion of NPC. Importantly, immune cells including macrophages, T cells, myeloid progenitors, and neutrophils were also identified, and further analysis showed that significant intercellular interaction between macrophages and Pro NPC occurred *via* MIF (macrophage migration inhibitory factor) and NF-kB signaling pathways during the progression of IDD. In addition, dynamic polarization of macrophage M1 and M2 cell subtypes was found in the progression of IDD, and gene set functional enrichment analysis suggested a significant role of the macrophage polarization in regulating cell metabolism, especially the Pro NPC. Finally, we found that the NP cells in the late degenerative stage were mainly composed of the cell types related to inflammatory and endoplasmic reticulum (ER) response, and fibrocartilaginous activity. Our results provided new insights into the identification of NP cell populations at single-cell resolution and at the relatively whole-transcriptome scale, accompanied by cellular communications between immune cells and NP cells, and discriminative markers in relation to specific cell subsets. These new findings present clues for effective and functional manipulation of human IDD-related bioremediation and healthcare.

Keywords: intervertebral disc degeneration, single-cell RNA sequencing, nucleus pulposus, gene, inflammation, metabolism

Research Reports: Biological

Transcriptomic Mapping of Human Parotid Gland at Single-Cell Resolution

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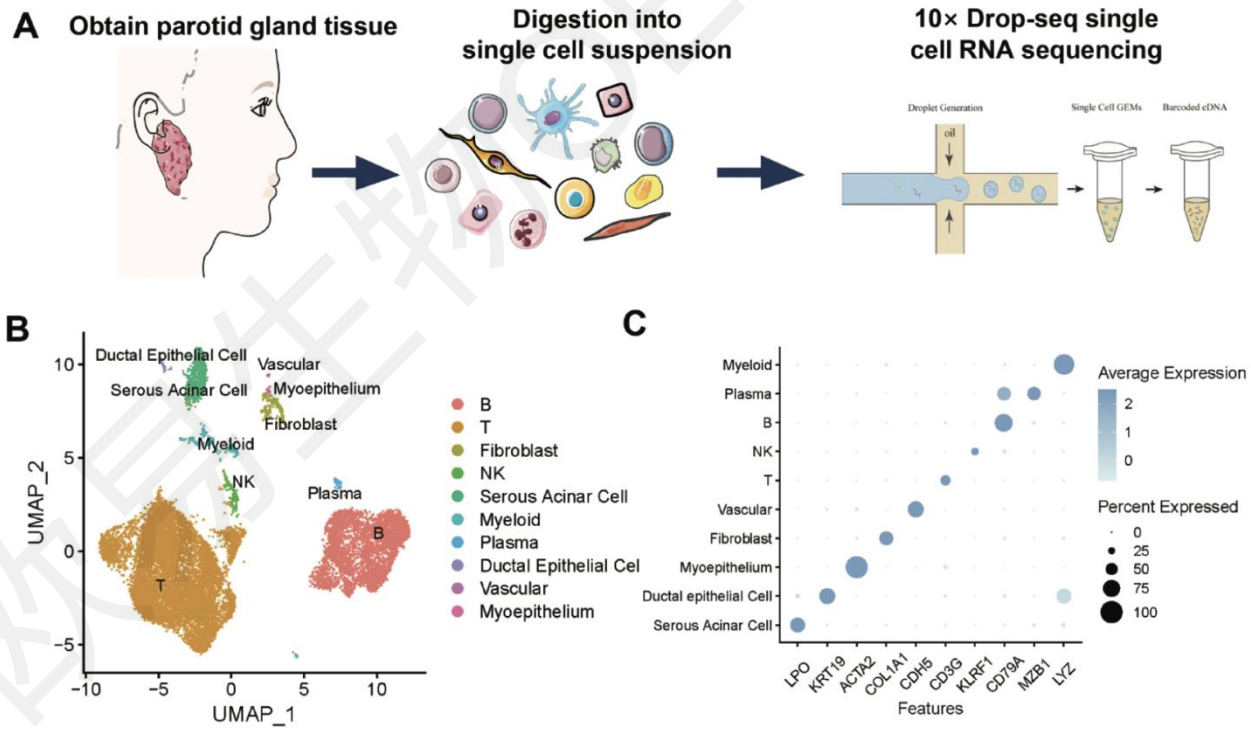
M. Chen[1,2*], W. Lin[1,3*], J. Gan[1,4], W. Lu[1,2], M. Wang[1], X. Wang[1,4], J. Yi[1,2], and Z. Zhao[1,2]

Abstract

As the largest salivary gland in oral cavity, the parotid gland plays an important role in initial digesting and lubricating food. The abnormal secretory function of the parotid gland can lead to dental caries and oral mucosal inflammation. In recent years, single-cell RNA sequencing (scRNA-seq) has been used to explore the heterogeneity and diversity of cells in various organs and tissues. However, the transcription profile of the human parotid gland at single-cell resolution has not been reported yet. In this study, we constructed the cell atlas of human parotid gland using the 10× Genomics platform. Characteristic gene analysis identified the biological functions of serous acinar cell populations in secreting digestive enzymes and antibacterial proteins. We revealed the specificity and similarity of the parotid gland compared to other digestive glands through comparative analyses of other published scRNA-seq data sets. We also identified the cell-specific expression of hub genes for Sjögren syndrome in the human parotid gland by integrating the results of genome-wide association studies and bulk RNA-seq, which highlighted the importance of immune cell dysfunction in parotid Sjögren syndrome pathogenesis.

Keywords: salivary glands, parotid gland, digestive system, single-cell RNA sequencing, bioinformatics, Sjögren syndrome



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Original Article

Integrated hepatic single-cell RNA sequencing and untargeted metabolomics reveals the immune and metabolic modulation of Qing-Fei-Pai-Du decoction in mice with coronavirus-induced pneumonia

Saisai Tian [a,1], Ningning Zheng [b,1], Xianpeng Zu [a,1], Gaosong Wu [b], Jing Zhong [b,c], Jinbo Zhang [a,d], Lili Sheng [b], Wei Liu [e], Chaoran Wang [f], Guangbo Ge [b], Jingyan Han [g], Jing Zhao [b], Houkai Li [b,*], Weidong Zhang [a,b,*]

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ABSTRACT

Background: Although Qing-Fei-Pai-Du decoction (QFPDD) is extensively used clinically to treat COVID-19 patients, the mechanism by which it modulates the immunological and metabolic functions of liver tissue remains unknown.

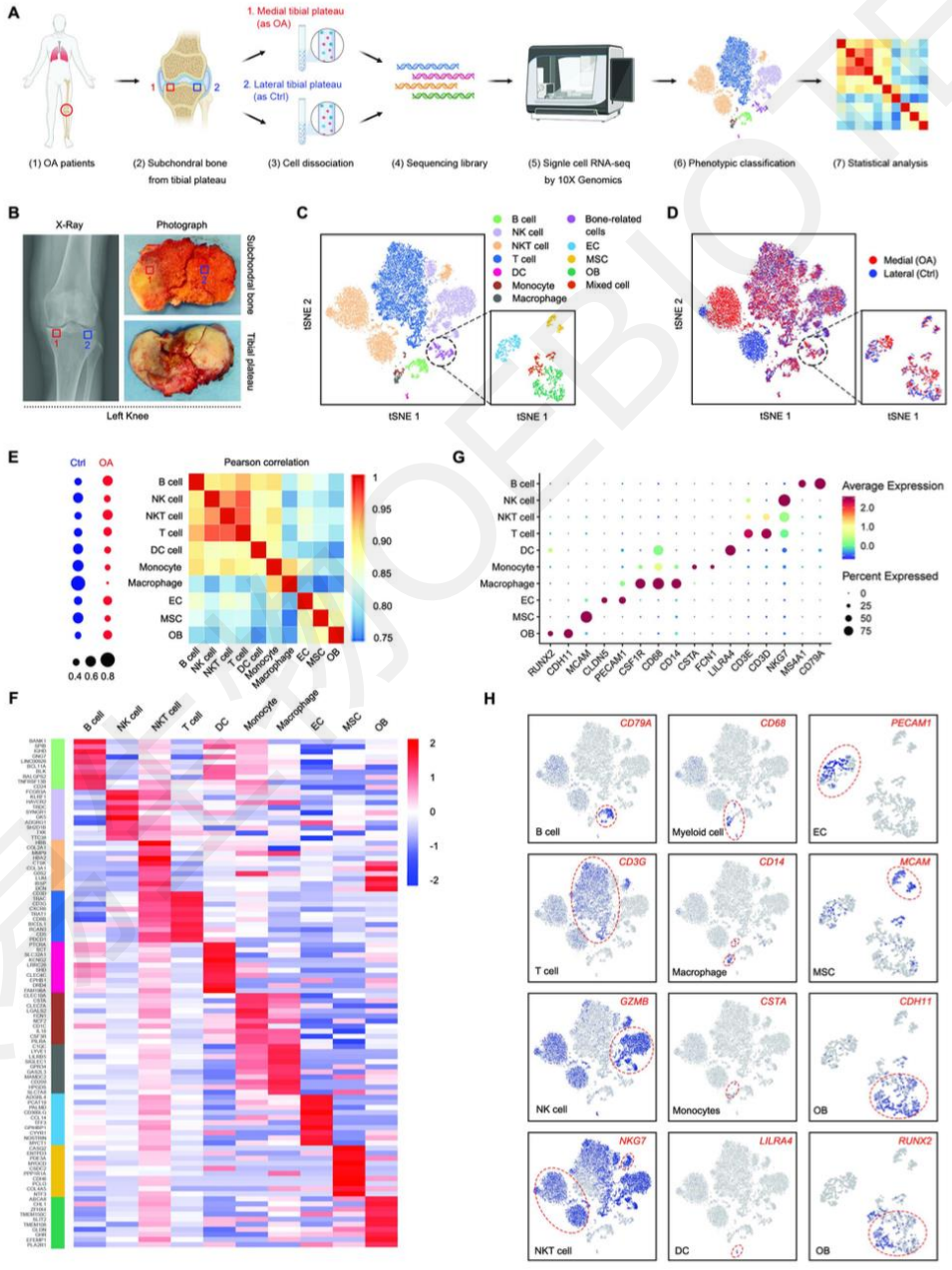
Purpose: The purpose of this study is to investigate the mechanism of action of QFPDD in the treatment of mice with coronavirus-induced pneumonia by combining integrated hepatic single-cell RNA sequencing and untargeted metabolomics.

Methods: We developed a human coronavirus pneumonia model in BALB/c mice by infecting them with human coronavirus HCoV-229E with stimulating them with cold-damp environment. We initially assessed the status of inflammation and immunity in model mice treated with or without QFPDD by detecting peripheral blood lymphocytes and inflammatory cytokines. Then, single-cell RNA sequencing and untargeted metabolomics were performed on mouse liver tissue.

Results: HCoV-229E infection in combination with exposure to a cold-damp environment significantly decreased the percentage of peripheral blood lymphocytes ($CD4^+$ and $CD8^+$ T cells, B cells) in mice, which was enhanced by QFPDD therapy. Meanwhile, the levels of inflammatory cytokines such as IL-6, TNF-α, and IFN-γ were significantly increased in mouse models but significantly decreased by QFPDD treatment. Single-cell RNA sequencing analysis showed that QFPDD could attenuate disease-associated alterations in gene expression, core transcriptional regulatory networks, and cell-type composition. Computational predictions indicated that QFPDD rectified the observed aberrant patterns of cell-cell communication. Additionally, the metabolic profiles of liver tissue in the Model group were distinct from mice in the Control group, and QFPDD significantly regulated hepatic purine metabolism.

Single-cell RNA-sequencing analysis reveals the molecular mechanism of subchondral bone cell heterogeneity in the development of osteoarthritis

Yan Hu[1], Jin Cui[2], Han Liu[1], Sicheng Wang[3], Qirong Zhou[2], Hao Zhang[2], Jiawei Guo[2], Liehu Cao[4*], Xiao Chen[2*], Ke Xu[1*], Jiacan Su[1,2*]



frontiers in Cell and Developmental Biology

ORIGINAL RESEARCH
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Single-Cell Transcriptomes Combining with Consecutive Genomics Reveal Clonal Evolution and Gene Regulatory Networks in Relapsed and Refractory Multiple Myeloma

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This study attempted to investigate how clonal structure evolves, along with potential regulatory networks, as a result of multiline therapies in relapsed/refractory multiple myeloma (RRMM). Eight whole exome sequencing (WES) and one single cell RNA sequencing (scRNA-seq) were performed in order to assess dynamic genomic changes in temporal consecutive samples of one RRMM patient from the time of diagnosis to death (about 37 months). The 63-year-old female patient who suffered from MM (P1) had disease progression (PD) nine times from July 2017 [newly diagnosed (ND)] to Aug 2020 (death), and the force to drive branching-pattern evolution of malignant PCs was found to be sustained. The mutant-allele tumor heterogeneity (MATH) and tumor mutation burden (TMB) initially exhibited a downward trend, which was then upward throughout the course of the disease. Various somatic single nucleotide variants (SNVs) that had disappeared after the previous treatment were observed to reappear in later stages. Chromosomal instability (CIN) and homologous recombination deficiency (HRD) scores were observed to be increased during periods of all progression, especially in the period of extramedullary plasmacytoma. Finally, in combination with WES and scRNA-seq of P1-PD9 (the nineth PD), the intro-heterogeneity and gene regulatory networks of MM cells were deciphered. As verified by the overall survival of MM patients in the MMRF CoMMpass and GSE24080 datasets, RUNX3 was identified as a potential driver for RRMM.

Keywords: multiple myeloma, clonal evolution, relapsed, refractory, heterogeneity

Title: Maintaining hypoxia environment of subchondral bone alleviates osteoarthritis progression

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- **Abstract:** Abnormal subchondral bone remodeling featured by over-activated osteoclastogenesis leads to articular cartilage degeneration and osteoarthritis (OA) progression, but the mechanism is 26 still unclear. In this study, we used lymphocyte cytosolic protein 1 (*Lcp1*) knock-out mice to suppress subchondral osteoclast formation in mice OA model with anterior cruciate ligament transection (ACLT) and *Lcp1*$^{-/-}$ mice showed decreased bone remodeling and sensory innervation in subchondral bone accompanied by retarded cartilage degeneration. For mechanisms, in wildtype mice with ACLT the activated osteoclasts in subchondral bone induced type-H vessels and elevated oxygen concentration which ubiquitylated hypoxia-inducible factor 1α (HIF-1α), vital for maintaining chondrocyte homeostasis in articular chondrocytes and led to cartilage degeneration. Deletion of *Lcp1* impeded osteoclast-mediated angiogenesis, which maintained the low levels of oxygen partial pressure (pO_2) in subchondral bone as well as the whole joint and delayed the OA progression. Stabilization of HIF-1α delayed cartilage degeneration and knockdown of *Hif1a* abolished the protective effects of *Lcp1* knockout. Notably, we identified a novel subgroup of hypertrophic chondrocytes highly associated with OA by single cell sequencing analysis of human articular chondrocytes. Lastly, we showed that Oroxylin A, an *Lcp1*-encoded protein L-plastin (LPL) inhibitor, could alleviate OA progression. In conclusion, maintaining hypoxic environment in subchondral bone is an attractive strategy for OA treatment.

- **Key words:** Osteoarthritis, Chondrocytes, Osteoclasts, angiogenesis, Hypoxia-Inducible Factor 1.

nature COMMUNICATIONS

ARTICLE

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Single-cell and spatial analysis reveal interaction of *FAP*+ fibroblasts and *SPP1*+ macrophages in colorectal cancer

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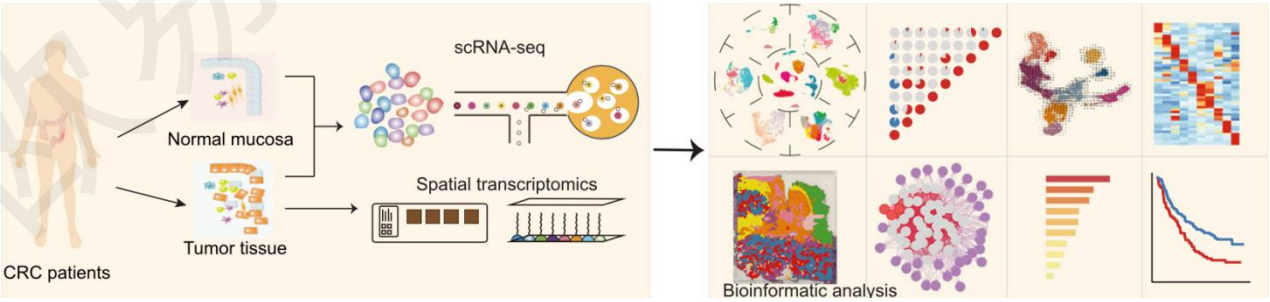
Abstract

Colorectal cancer (CRC) is among the most common malignancies with limited treatments other than surgery. The tumor microenvironment (TME) profiling enables the discovery of potential therapeutic targets. Here, we profile 54,103 cells from tumor and adjacent tissues to characterize cellular composition and elucidate the potential origin and regulation of tumor- enriched cell types in CRC. We demonstrate that the tumor-specific FAP^+ fibroblasts and $SPP1^+$ macrophages were positively correlated in 14 independent CRC cohorts containing 2550 samples and validate their close localization by immuno-fluorescent staining and spatial transcriptomics. This interaction might be regulated by chemerin, TGF-β, and interleukin-1, which would stimulate the formation of immune-excluded desmoplasic structure and limit the T cell infiltration. Furthermore, we find patients with high FAP or SPP1 expression achieved less therapeutic benefit from an anti-PD-L1 therapy cohort. Our results provide a potential therapeutic strategy by disrupting FAP^+ fibroblasts and $SPP1^+$ macrophages interaction to improve immunotherapy.



DOI: 10.1002/ctm2.800

RESEARCH ARTICLE

CLINICAL AND TRANSLATIONAL MEDICINE WILEY

Hepatocellular carcinoma-infiltrating γδ T cells are functionally defected and allogenic Vδ2$^+$ γδ T cell can be a promising complement

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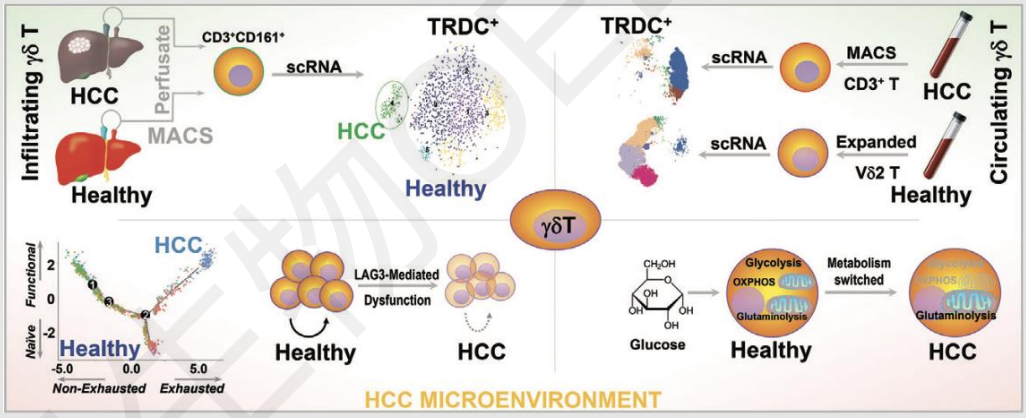
Graphical Abstract



(1) HCC-infiltrating γδ T cells lost T cell receptor (TCR) diversity and were G2/M cell cycle arrested.
(2) γδ T cell dysfunction in HCC was LAG3 dependent.
(3) Glutamine metabolism-mediated γδ T cell dysfunction in HCC.
(4) Allogeneic Vδ2$^+$ γδ T cells can complement functional deficiency of γδ T cells in HCC.

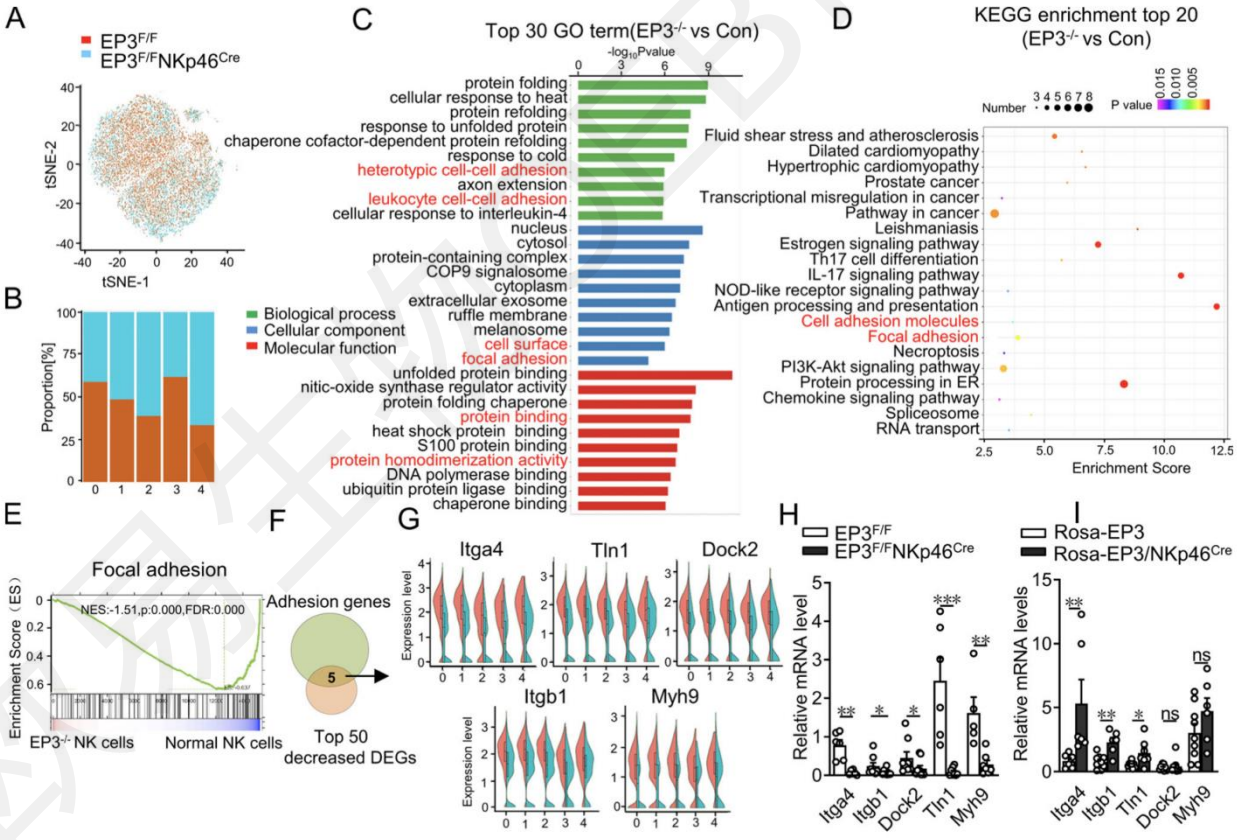
JEM Journal of Experimental Medicine

ARTICLE

EP3 enhances adhesion and cytotoxicity of NK cells toward hepatic stellate cells in a murine liver fibrosis model

Xixi Tao[1*], Rui Zhang[1*], Ronglu Du[1*], Tingting Yu[1*], Hui Yang[2], Jiwen Li[2], Yuhong Wang[1], Qian Liu[1], Shengkai Zuo[1], Xi Wang[3], Michael Lazarus[4], Lu Zhou[2], Bangmao Wang[2], Ying Yu[1], and Yujun Shen[1]

Natural killer (NK) cells exhibit antifibrotic properties in liver fibrosis (LF) by suppressing activated hepatic stellate cell (HSC) populations. Prostaglandin E_2 (PGE_2) plays a dual role in innate and adaptive immunity. Here, we found that E-prostanoid 3 receptor (EP3) was markedly downregulated in NK cells from liver fibrosis mice and patients with liver cirrhosis. NK cell–specific deletion of EP3 aggravated hepatic fibrogenesis in mouse models of LF. Loss of EP3 selectively reduced the cytotoxicity of the $CD27^+CD11b^+$ double positive (DP) NK subset against activated HSCs. Mechanistically, deletion of EP3 impaired the adhesion and cytotoxicity of DP NK cells toward HSCs through modulation of Itga4-VCAM1 binding. EP3 upregulated Itga4 expression in NK cells through promoting Spic nuclear translocation via PKC-mediated phosphorylation of Spic at T191. Activation of EP3 by sulprostone alleviated CCL4-induced liver fibrosis in mice. Thus, EP3 is required for adhesion and cytotoxicity of NK cells toward HSCs and may serve as a therapeutic target for the management of LF.



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Journal of Translational Medicine

RESEARCH Open Access

Cisplatin resistance-related multi-omics differences and the establishment of machine learning models

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Abstract

Objectives: Platinum-based chemotherapies are currently the first-line treatment of non-small cell lung cancer. This study will improve our understanding of the causes of resistance to cisplatin, especially in lung adenocarcinoma (LUAD) and provide a reference for therapeutic decisions in clinical practice.

Methods: Cancer Cell Line Encyclopedia (CCLE), The Cancer Genome Atlas (TCGA) and Zhongshan hospital affiliated to Fudan University (zs-cohort) were used to identify the multi-omics differences related to platinum chemotherapy. Cisplatin-resistant mRNA and miRNA models were constructed by Logistic regression, classification and regression tree and C4.5 decision tree classification algorithm with previous feature selection performed via least absolute shrinkage and selection operator (LASSO). qRT-PCR and western-blotting of A549 and H358 cells, as well as single-cell Seq data of tumor samples were applied to verify the tendency of certain genes.

Results: 661 cell lines were divided into three groups according to the IC50 value of cisplatin, and the top 1/3 (220) with a small IC50 value were defined as the sensitive group while the last 1/3 (220) were enrolled in the insensitive group. TP53 was the most common mutation in the insensitive group, in contrast to TTN in the sensitive group. 1348 mRNA, 80 miRNA, and 15 metabolites were differentially expressed between 2 groups ($P < 0.05$). According to the LASSO penalized logistic modeling, 6 of the 1348 mRNAs, FOXA2, BATF3, SIX1, HOXA1, ZBTB38, IRF5, were selected as the associated features with cisplatin resistance and for the contribution of predictive mRNA model (all of adjusted P-values < 0.001). Three of 6 (BATF3, IRF5, ZBTB38) genes were finally verified in cell level and patients in zs-cohort.

Conclusions: Somatic mutations, mRNA expressions, miRNA expressions, metabolites and methylation were related to the resistance of cisplatin. The models we created could help in the prediction of the reaction and prognosis of patients given platinum-based chemotherapies.

Keywords: Cisplatin, Drug resistance, Machine learning, IC50

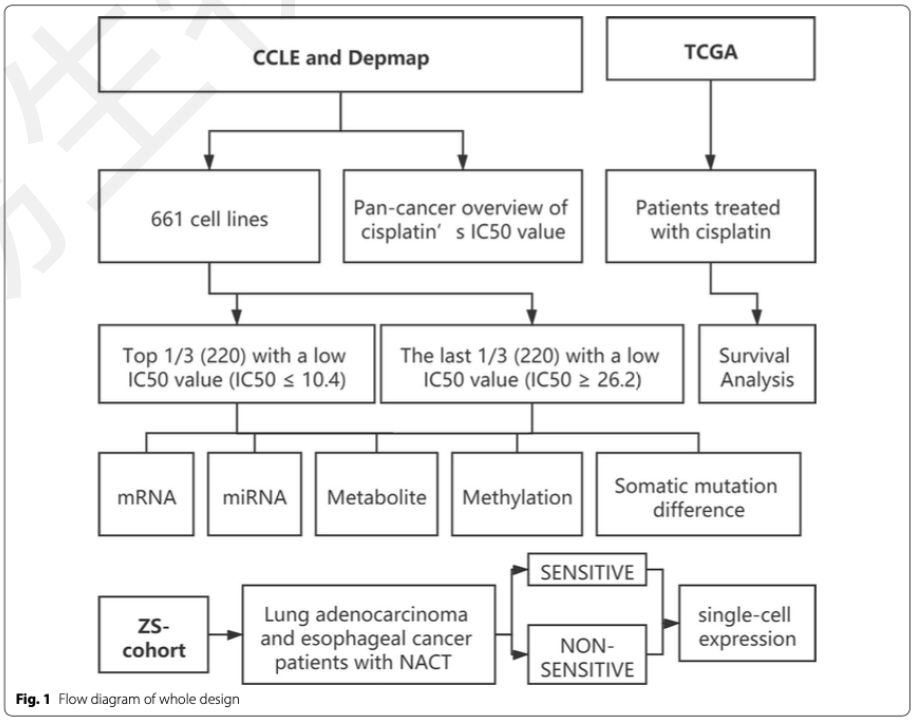

Fig. 1 Flow diagram of whole design

Molecular Therapy
Nucleic Acids
Original Article

miR-6077 promotes cisplatin/pemetrexed resistance in lung adenocarcinoma via CDKN1A/ cell cycle arrest and KEAP1/ferroptosis pathways

Guoshu Bi,[1,2,3] Jiaqi Liang,[1,2,3] Mengnan Zhao,[1,2,3] Huan Zhang,[1,2] Xing Jin,[1,2] Tao Lu,[1,2] Yuansheng Zheng,[1,2] Yunyi Bian,[1,2] Zhencong Chen,[1,2] Yiwei Huang,[1,2] Valeria Besskaya,[1,2] Cheng Zhan,[1,2] Qun Wang,[1,2] and Lijie Tan[1,2]

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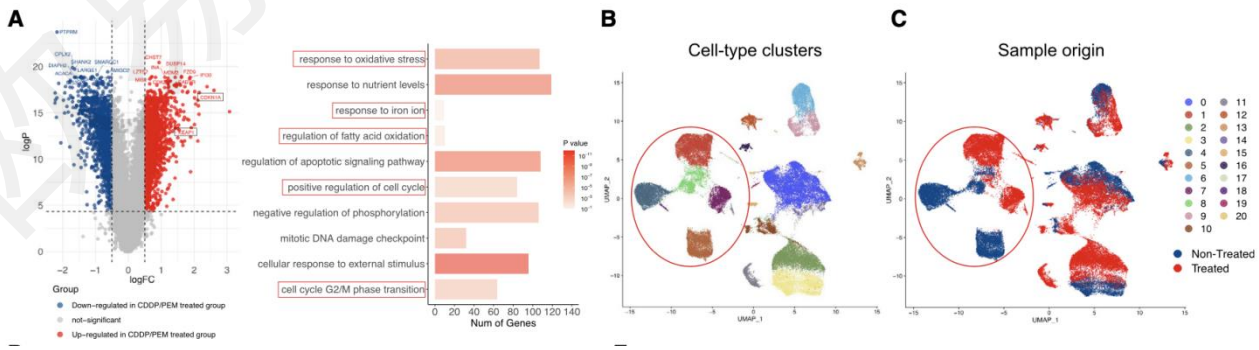
Lung adenocarcinoma (LUAD) is one of the most common malignancies worldwide. Combination chemotherapy with cisplatin (CDDP) plus pemetrexed (PEM) remains the predom- inant therapeutic regimen; however, chemoresistance greatly limits its curative potential. Here, through CRISPR-Cas9 screening, we identified miR-6077 as a key driver of CDDP/ PEM resistance in LUAD. Functional experiments verified that ectopic overexpression of miR-6077 desensitized LUAD cells to CDDP/PEM in both cell lines and patient-derived xeno- graft models. Through RNA sequencing in cells and single-cell sequencing of samples from patients with CDDP/PEM treat- ments, we observed CDDP/PEM-induced upregulation of CDKN1A and KEAP1, which in turn activated cell-cycle arrest and ferroptosis, respectively, thus leading to cell death. Through miRNA pull-down, we identified and validated that miR-6077 targets CDKN1A and KEAP1. Furthermore, we demonstrated that miR-6077 protects LUAD cells from cell death induced by CDDP/PEM via CDKN1A-CDK1-mediated cell-cycle arrest and KEAP1-NRF2-SLC7A11/NQO1-mediated ferroptosis, thus resulting in chemoresistance in multiple LUAD cells both in vitro and in vivo. Moreover, we found that GMDS-AS1 and LINC01128 sensitized LUAD cells to CDDP/PEM by sponging miR-6077. Collectively, these results imply the critical role of miR-6077 in LUAD's sensitivity to CDDP/PEM, thus providing a novel therapeutic strategy for overcoming chemoresistance in clinical practice.



frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
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Single-Cell Sequencing of Immune Cells in Human Aortic Dissection Tissue Provides Insights Into Immune Cell Heterogeneity

OPEN ACCESS

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Background: Inflammation plays an important role in the progression of sporadic aortic dissection (AD). Immune cells, especially macrophages, infiltrate the aorta and secrete inflammatory cytokines and matrix metalloproteinases to cause degradation of the extracellular matrix, thereby contributing to the pathogenesis of AD. However, the cellular heterogeneity within these immune cells has not been fully characterized.

Methods: We used single-cell RNA sequencing to profile the transcriptomes of all immune cells in AD tissue and normal aorta. Using magnetic-activated cell sorting gating on CD45, we obtained a higher resolution identification of the immune cell subsets in the aorta.

Results: We observed significant differences in the proportion of major immune cell subpopulations between AD and normal aorta tissues. Macrophages accounted for a higher percentage in the normal aorta, while the proportions of T cells, B cells and natural killer (NK) cells were all increased in AD tissues. Macrophage clusters that expanded in AD tissues originated primarily from circulating monocytes and expressed genes encoding proinflammatory cytokines and molecules involved in tissue repair. T and NK cells in AD tissues exhibited enhanced cytotoxic properties. A cluster of $CD4^+$ T cells that had expanded in AD tissues was Th17-like and might contribute to the pathogenesis of AD. Cell–cell interaction analysis highlighted the increased communication between macrophages and T cells, which primarily regulated the costimulation of T cells.

Conclusions: Our study provides a comprehensive characterization of immune cells in the dissected aorta with an emphasis on the role of macrophages and T cells. The information from our study improves our understanding of immune mechanisms in AD formation and helps to identify additional useful targets for early diagnosis or therapy of AD.

Keywords: single-cell RNA sequencing, aortic dissection, immune cell heterogeneity, T cells, macrophages, leukocyte

SCIENCE CHINA Life Sciences

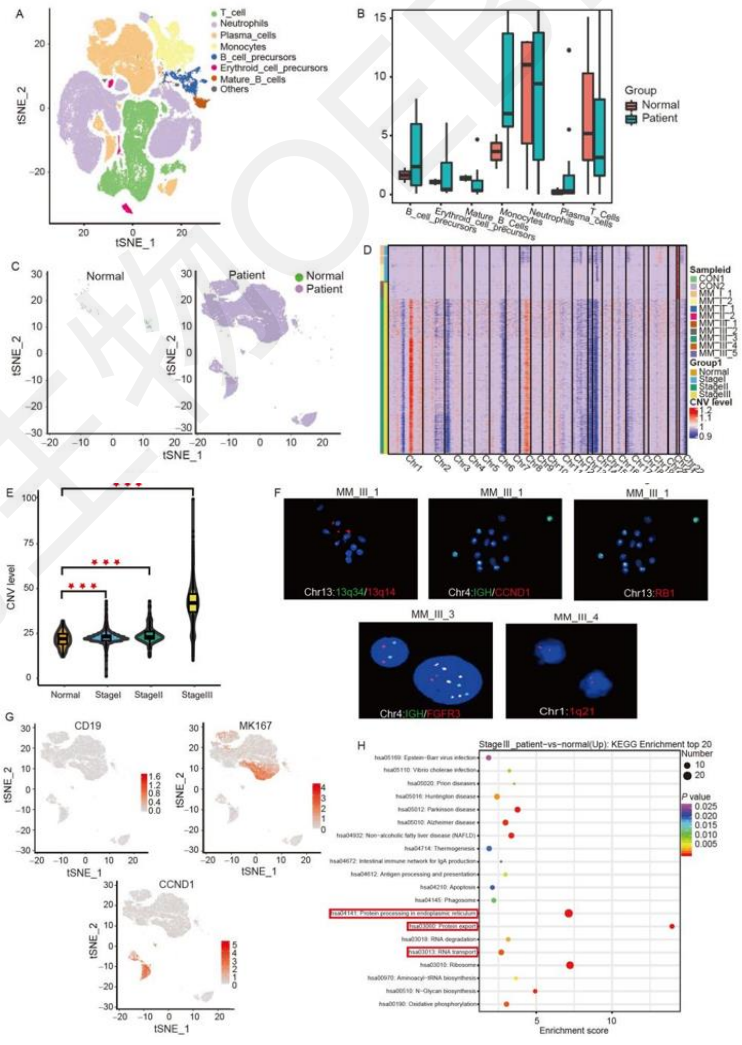
Exploring the R-ISS stage-specific regular networks in the progression of multiple myeloma at single-cell resolution

Ling Zhong[1,2,3,4,†], Xiao Yang[5,†], Yu Zhou[1,2,3,†], Jialing Xiao[1,†], Huan Li[1], Jiang Tao[6], Qian Xi[1], Chen Chu[7], Chenglong Li[6], Xi Yang[6], Chen Yang[1], Yi Zhang[1], Ping Shuai[1], Yuping Liu[1], Man Yu[2], Yi Shi[1,2,3], Jiang Hu[5], Wei Zhang[5], Bo Gong[1,2,3,*] and Zhenglin Yang[2,3,*]

Citation: SCIENCE CHINA Life Sciences; doi: 10.1007/s11427-021-2097-1

The Revised International Staging System (R-ISS) is a simple and powerful prognostic tool for multiple myeloma (MM). However, heterogeneity in R-ISS stage is still poorly characterised, hampering improvement of treatments. We used single-cell RNA-seq to examine novel cellular heterogeneity and regular networks in nine MM patients stratified by R-ISS. Plasma cells were clustered into nine groups (P1–P9) based on gene expression, where P1–P5 were almost enriched in stage III.*PDIA6* was significantly upregulated in P3 and *LETM1*was enriched in P1, and they were validated to be upregulated in the MM cell line and in 22 other patients' myeloma cells. Furthermore, in progression, *PDIA6* was newly found and verified to be activated by *UQCRB* through oxidative phosphorylation, while *LETM1* was activated by *STAT1* via the C-type lectin receptor-signalling pathway. Finally, a subcluster of monocytes was exclusively found in stage III specifically expressed chemokines modulated by *ATF3*. A few ligand-receptor pairs (*CCL3*/*CCL5*/*CCL3L1*-*CCR1*) were obviously active in monocyte-plasma communications in stage III. Herein, this study identified novel molecules, networks and crosstalk pairs in different R-ISS stages of MM, providing significant insight for its prognosis and treatment.

multiple myeloma, the revised international staging system, single-cell RNA-seq



RESEARCH ARTICLE

ADVANCED SCIENCE Open Access
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Single-Cell RNA Sequencing Reveals Heterogeneity of Myf5-Derived Cells and Altered Myogenic Fate in the Absence of SRSF2

Ruochen Guo, Xue You, Kai Meng, Rula Sha, Zhenzhen Wang, Ningyang Yuan, Qian Peng, Zhigang Li, Zhiqin Xie, Ruijiao Chen, and Ying Feng**

Splicing factor SRSF2 acts as a critical regulator for cell survival, however, it remains unknown whether SRSF2 is involved in myoblast proliferation and myogenesis. Here, knockdown of SRSF2 in myoblasts causes high rates of apoptosis and defective differentiation. Combined conditional knockout and lineage tracing approaches show that Myf5-cre mice lacking SRSF2 die immediately at birth and exhibit a complete absence of mature myofibers. Mutant Myf5-derived cells (tdtomato-positive cells) are randomly scattered in the myogenic and non-myogenic regions, indicating loss of the community effect required for skeletal muscle differentiation. Single-cell RNA-sequencing reveals high heterogeneity of myf5-derived cells and non-myogenic cells are significantly increased at the expense of skeletal muscle cells in the absence of SRSF2, reflecting altered cell fate. SRSF2 is demonstrated to regulate the entry of Myf5 cells into the myogenic program and ensures their survival by preventing precocious differentiation and apoptosis. In summary, SRSF2 functions as an essential regulator for Myf5-derived cells to respond correctly to positional cues and to adopt their myogenic fate.

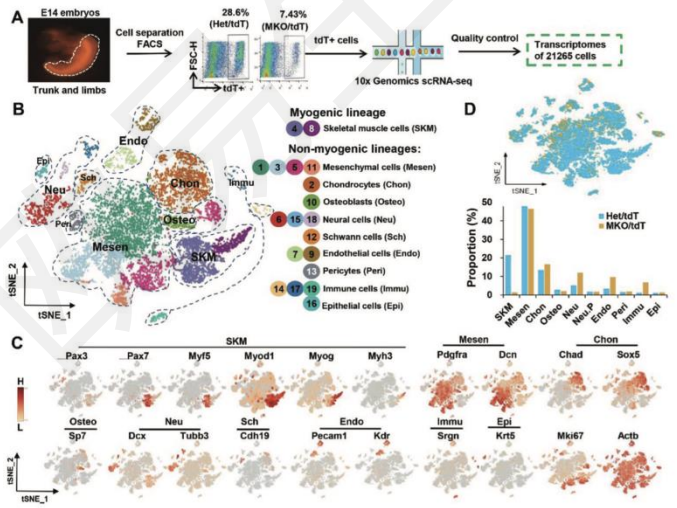

Figure 5. scRNA-seq identifies distinct cell lineages of Myf5-derived cells. A) Scheme of single cell preparation from control and MKO/tdT truncated embryos at E14, FACS isolation for tdT+ cell, and scRNA-seq. B) t-SNE plot of combined dataset with cells colored by cell types. C) Expression of representative marker genes in distinct cell clusters. D) t-SNE plot of overlapping control and MKO/tdT datasets (top), and the proportion of different cell types in control and MKO/tdT samples (bottom).

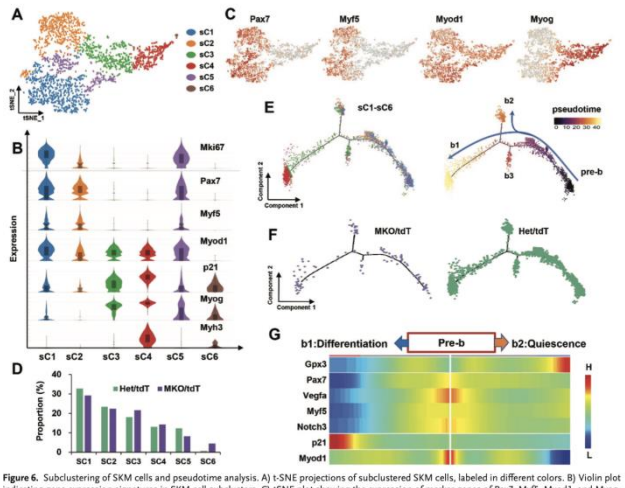

Figure 6. Subclustering of SKM cells and pseudotime analysis. A) t-SNE projections of subclustered SKM cells, labeled in different colors. B) Violin plot indicating gene expression signatures in SKM cell subclusters. C) tSNE plot showing the expression of marker genes of Pax7, Myf5, Myod1, and Myog. D) the proportion of subclusters in control and MKO/tdT samples. E) Pseudotime-ordered analysis of SKM cells, constructed by Monocle2. Right panel was colored by the subclusters. Left panel was colored by the pseudotime order. F) Pseudotime-ordered analysis of SKM cells, from the mutant (left panel) and control (right panel) samples. G) Heatmap showing the dynamic expression of the selected genes along the trajectories b1 and b2.

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Mesenchymal stem cell treatment restores liver macrophages homeostasis to alleviate mouse acute liver injury revealed by single-cell analysis

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ARTICLE INFO

Keywords:
Mesenchymal stem cells
Acute liver injury
Single-cell RNA sequencing
Monocyte
Monocyte-derived macrophages

Chemical compounds studied in this article:
Carbon tetrachloride (PubChem CID: 5943)
Dimethyl sulfoxide (PubChem CID: 679)
4', 6-Diamidino-2-phenylindole (PubChem CID: 2954)

ABSTRACT

Acute liver injury (ALI) is characterized by massive hepatocyte necrosis and subsequent recruitment of myeloid cells to liver. Mesenchymal stem cells (MSCs) have therapeutic potential for ALI through their immunoregulation on macrophages, but the mechanism is not completely clear due to the heterogeneity and controversy of liver macrophages. Here, we detected the survival rate, biochemical indexes, histopathology, and inflammatory chemokine levels to assess the efficacy of MSC treatment on CCl_4-induced ALI of C57BL/6 mice. Furthermore, flow cytometry and single-cell RNA sequencing (scRNA-Seq) were used to precisely distinguish macrophage populations and reveal the immunoregulation of MSCs. MSC treatment could effectively alleviate ALI and mitigate the recruitment of mononuclear phagocytes. Flow cytometry and scRNA-Seq analyses collectively indicated that there were monocytes with high Ly6C expression and heterogeneous monocyte-derived macrophages (MoMF) with low Ly6C expression in liver. $Ly6C^{hi}$ pro-inflammatory monocytes and $Ly6C^{lo}$ MoMF with powerful phagocytosis dominated during the acute injury period. MSC treatment promoted the transition from $Ly6C^{hi}$ to $Ly6C^{lo}$ population, inhibit the proinflammatory function of monocytes and promote the lysosomal function of MoMF. Furthermore, MSCs attenuated the recruitment of neutrophils by reducing the expression of CXCL2 of MoMF. MoMF with high expression of arginase 1 appeared during the recovery period, and MSCs could increase their expression of arginase 1, which may promote liver repair. To sum up, we demonstrated the characteristics of distinct MoMF during different periods of ALI and revealed their functional changes after MSC treatment, providing immunotherapeutic targets for MSC treatment of ALI.

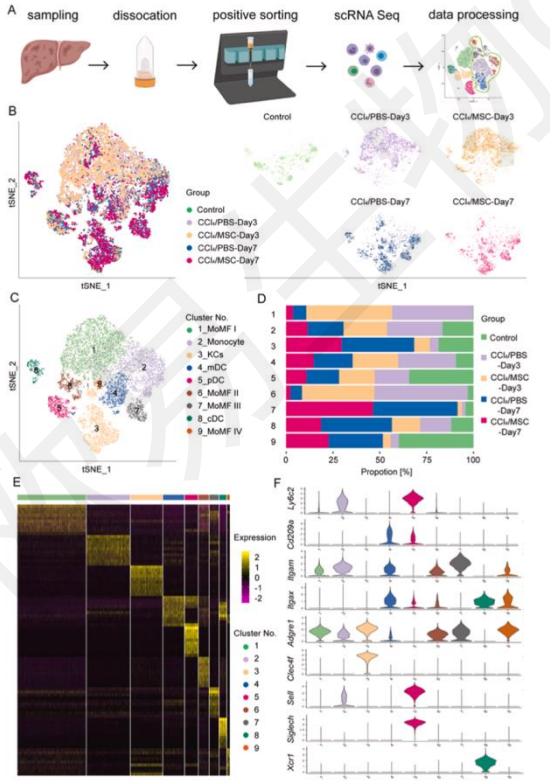

Fig. 4. scRNA-seq analysis of the mononuclear phagocyte system in liver. (A) The workflow of scRNA-seq including cell isolation of the liver, magnetically activated cell sorting, and data processing. (B, C) t-Distributed Stochastic Neighbor Embedding (t-SNE) plot to display scRNA-seq data of selected mononuclear phagocyte system from the liver exhibited by groups (B) and cell types (C). (D) The proportion of nine cell subgroups in five groups. (E) Top 10 DEGs among nine clusters of the mononuclear phagocyte system. (F) Violin plots of the relative expression of marker genes for each cluster.

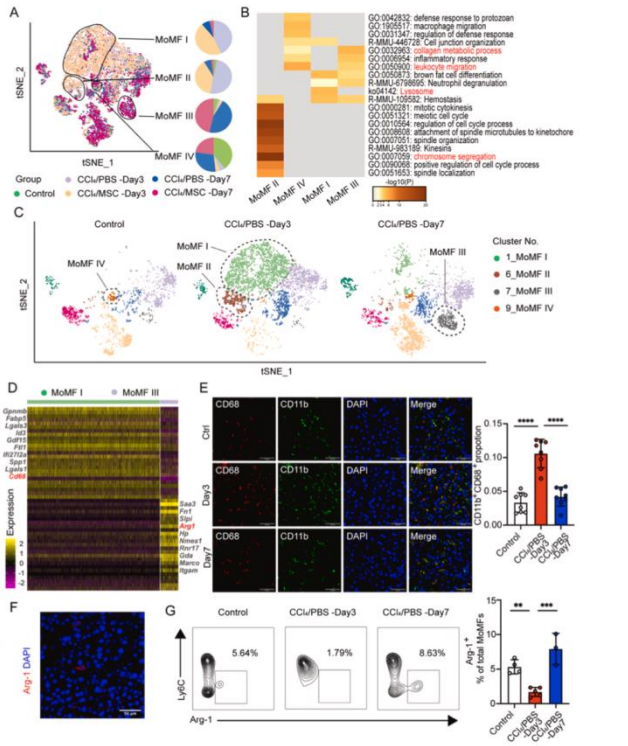

Fig. 5. Characteristics of the four MoMF subsets in liver. (A) t-SNE plot with cell identities and the relative abundance as a pie chart of each cluster depending on the three groups with two time points. (B) Heatmap of the top 50 genes ontology (GO) enrichment of four MoMF subsets. (C) t-SNE plot of clusters in Control group and CCl_4/PBS group on day 3 and day 7. (D) Heatmap of top 25 DEGs of MoMF I and MoMF III. (E) Representative immunofluorescent (IF) staining images of CD68 (red) and CD11b (green) in the liver, counterstained by DAPI (blue) in the CCl_4/PBS group on day 3. N = 8 per group. Scale bars, 50 μm. (F) IF staining images of Arg1 (red) in liver, counterstained by DAPI (blue) in the CCl_4/PBS group on day 7. Scale bars, 50 μm. (G) Representative and quantitative flow cytometric analysis of Arg1 expression in the recruited mononuclear phagocytes. The red line represented the isotype of Arg1 and the blue part represented the expression of Arg1. N = 3–5 per group. All data were shown as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Representative flow cytometry plots, histograms, and images are shown.

单细胞事业部 / 欧易生物

SINGLE CELL
MULTIOMICS

欧易生物单细胞事业部成立于2018年，是欧易生物最为核心的部门之一。自成立以来，陆续引入多套10x Genomics、BD Rhapsody™等主流高通量单细胞分选平台，流式细胞分选系统和美天旎全自动样本处理仪，同时获得10x Genomics授权的单细胞基因表达和空间转录组双平台认证。目前已在北京、广州等地协同科研院所成立联合实验室。

单细胞测序平台拥有超1000㎡的标准实验室和办公区，可高质量完成样本处理、细胞分选、文库构建和空间转录等实验。已累计完成1000+个服务项目、8000+例样本的单细胞解离与测序。样本类型涵盖人、大/小鼠、羊、兔、猴、鱼、鸡、蟹、蜘蛛等400余种不同的动物组织类型以及拟南芥、水稻、玉米、棉花、大豆、小麦、番茄、茶树、桑树、杨树及药用植物等50余种植物样本。

截止目前，单细胞事业部已累计参与/协助客户发表60+篇高水平文章，影响因子600+，其中致谢公司26次，致谢团队18次，作者署名2人。

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