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ORIGINAL ARTICLE

# Single-cell RNA sequencing reveals the heterogeneity and microenvironment in one adenoid cystic carcinoma sample

Pu-Gen An[1,2,3] · Wen-Jie Wu[1,2,3] · Yu-Fang Tang[1,2,3,4] · Jie Zhang[1,2,3]

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## Abstract

Adenoid cystic carcinoma (ACC) is one of the most common malignancy of the major salivary glands with a high recurrence rate and poor prognosis. Determining tumor heterogeneity and factors in the microenvironment may provide novel therapeutic targets for ACC. We performed single-cell RNA sequencing of one ACC sample and normal salivary gland tissues from a patient to analyze tumor heterogeneity, immunosuppressive landscape, and intercellular communication networks. The heterogeneity of epithelial cells in ACC tissues was significantly higher compared with that in normal tissues, whereas immune cells were almost absent. We found four malignant cell clusters in ACC and explored their characteristics and function. In tumor tissues, CD8 + cytotoxic T cells and CD4 + T helper cells were significantly decreased, whereas IgA + plasma cells were absent. There were two clusters of macrophages, one representing IL1B macrophages and the other consisted of a cluster of macrophages associated with the epithelial mesenchymal transition (EMT). Both were significantly different from the normal tissue composition. In addition, the communication between epithelial cells and other cells in the tumor tissue was enhanced. MIF-CD74 and APP-CD74 were significantly upregulated. We comprehensively described the heterogeneity of ACC and the tumor microenvironment (TME) from a single cell perspective including cell characteristics, immune cell infiltration, and cell communication.

**Clinical relevance** This study provided further insights into ACC and may lead to new treatment strategies.

**Keywords** Adenoid cystic carcinoma · Single-cell RNA sequencing · Tumor heterogeneity · Tumor microenvironment

## Introduction

ACC is one of the most common malignant tumors of the major salivary glands. It exhibits strong local invasiveness, distant metastasis tendency, and readily grows along the nerve (Coca-Pelaz et al. 2015; Ho et al. 2013). Chromosome 6:9 translocations in ACC generate an MYB:NFIB gene fusion resulting in overexpression of the MYB oncoprotein (Ho et al. 2013; Persson et al. 2009). The tumor is difficult to completely remove by surgery, has a high recurrence rate, and is insensitive to radiotherapy, resulting in an extremely low survival rate (Jensen et al. 2015). Although Lenvatinib (a multi-target tyrosine kinase inhibitor) has been evaluated in a phase II clinical trial of recurrent/metastatic ACC, only 15.6% of patients experience a partial remission (Tchekmedyian et al. 2019). EGFR, c-kit, and other genes are highly expressed in ACC; however, drugs designed to interact with these targets have yielded disappointing results (Laurie et al. 2011). Thus far, the treatment of ACC remains a great challenge. Therefore, it is necessary to further understand tumor

Pu-Gen An and Wen-Jie Wu contributed equally to this work.

✉ Jie Zhang
zhangjie06@126.com

1 Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, No. 22 Zhongguancun South Avenue, Beijing 100081, People's Republic of China

2 National Center of Stomatology & National Clinical Research Center for Oral Diseases, Beijing 100081, People's Republic of China

3 Central Laboratory, Peking University School and Hospital of Stomatology, Beijing 100081, People's Republic of China

4 Department of Stomatology, Xinqiao Hospital (the Second Affiliated Hospital), Army Medical University, Chongqing 400037, People's Republic of China

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heterogeneity and the microenvironment of ACC to develop effective treatments.

Single-cell sequencing represents a technological revolution. Its development has greatly facilitated studies on tumor heterogeneity and it may be used to analyze the TME, tumor immune invasion, and epithelial mesenchymal transition (EMT) (Cillo et al. 2020; Chung et al. 2017; Darmanis et al. 2017). In this study, we comprehensively analyzed the heterogeneity and microenvironment of an ACC tumor sample using single-cell sequencing technology. The results provide insight into the heterogeneity and microenvironment of ACC.

## Materials and methods

### Sample preparation

The patient is a 42-year-old male with a left palate tumor of 1.5 cm × 1.5 cm in size. He underwent extended tumor resection and left neck dissection. The pathology of the tumor was tubular and cribriform type. The ACC sample was obtained from resected tumor tissue and the normal tissue was the submandibular gland. Single-cell isolation was performed and varied depending on the sample conditions. Tumor and normal tissue processing were done using a tumor dissociation kit (Miltenyi Biotech, Germany) following the manufacturer's instructions. The cells were washed in a MACS SmartStrainer (30 µm or 70 µm) with 20 mL of RPMI 1640 and the cell suspension was centrifuged at 300 × g for 7 min. The supernatant was aspirated and the cells were resuspended as required for the experiments.

### Single-cell RNA sequencing and read processing

Each cell suspension was subjected to 3′ single-cell RNA sequencing using a Chromium Next GEM Chip G Single Cell Kit, Single Cell 3′ Library, and Gel Bead Kit V3.1 (10 × Genomics) following the manufacturer's instructions. Libraries were sequenced on an Illumina Novaseq6000 and mapped to the GRCh38 human reference genome using the Cell Ranger toolkit (version 4.0.0).

### Immunofluorescence

Paraffin-embedded ACC were sectioned coronally into 4 µm slices. They were deparaffinized, rehydrated, and antigen retrieval was done by heating in 0.01 M citric acid. The slides were rinsed in 0.01 M PBS, incubated with 0.1% Triton X-100 for 15 min, and blocked with 5% bovine serum albumin for 30 min at room temperature. The slides were then incubated with the following primary antibodies overnight at 4℃: NTRK3 (1:250, 3376S, CST), BAMBI (1:50, ab203070, Abcam), TNFRSF12A (1:400, PA5-109,244, Thermo), and EFNA5 (1:400, PA5-47,532, Thermo). After washing three times in 0.01 M PBS, the slides were incubated with the following secondary antibodies for 1 h at 37℃: donkey antirabbit lgG (H + L) AlexaFluor®555 (1:1000, A32794, technologies), or HRP-labeled goat antirabbit IgG (1:4000, ab205718, Abcam). Finally, the slides were cover-slipped with antifade mounting medium and photographed using an Olympus #BX53 microscope with the same parameters.

### Analysis of scRNA-seq data

The following criteria were used to identify and discard poor-quality cell data: (1) if the number of total UMI counts per cell was more than the mean value plus twofold of the standard deviation or less than 500; (2) if the number of genes detected was more than the mean value plus twofold of the standard deviation or less than 400 and genes were discarded that were expressed in < 1% cells; and (3) if the proportion of reads mapping to mitochondrial genes was > 10%. Seurat was then used to normalize the data to sequencing depth using a LogNormalize implementation, whereas mitochondrial contamination and cell cycle effects were removed. A subset of significant PCs was selected using the PCElbowPlot function of Seurat. Cell clustering and t-SNE visualization were performed using the FindClusters and RunTSNE functions, respectively. The annotations for the cell identity of each cluster were defined by package singleR and the expression of known marker genes. For analysis of normal control data and normal control and tumor data, doublets detected by Scrublet, cells with lower than 500 genes and cells with a mitochondrial percentage greater than 15% were filtered. Seurat was used to perform downstream cell clustering and differential gene expression. Differentially expressed genes were tested by the Wilcoxon rank sum test and P values were corrected by Bonferroni's method. An adjusted P value less than 0.05 was considered statistically significant. CopyKAT was used to assess copy number variation and predict tumor and normal cells. Over representation pathway analysis of GO ontology biological processes was performed by ClusterProfiler and P values were corrected by the Benjamini–Hochberg method, with q values less than 0.05 considered statistically significant. Gene sets were downloaded from the Molecular Signatures Database to assess module scores in cell clusters using the AddModuleScore function in Seurat. CellChat was used to analyze cell–cell communication using default settings. Table S1 shows the quality control of the single-cell RNA sequencing of this study.

## Results

### Cluster analysis of normal gland cells

First, we analyzed the normal submandibular gland cells. After quality control, we obtained 1376 cells (Fig. S1A). To visualize the sequenced single-cell gene map, we divided the cells into 12 different cell types using unsupervised cluster analysis and specific cell markers. In normal submandibular gland tissues, the number of epithelial cells and T cells was the largest (Fig. 1A–B). Figure 1C shows the classic genes that defined the cell population displayed, such as epithelial cells, T cells, and mesenchymal cells. Different types of cell cluster expressed their own

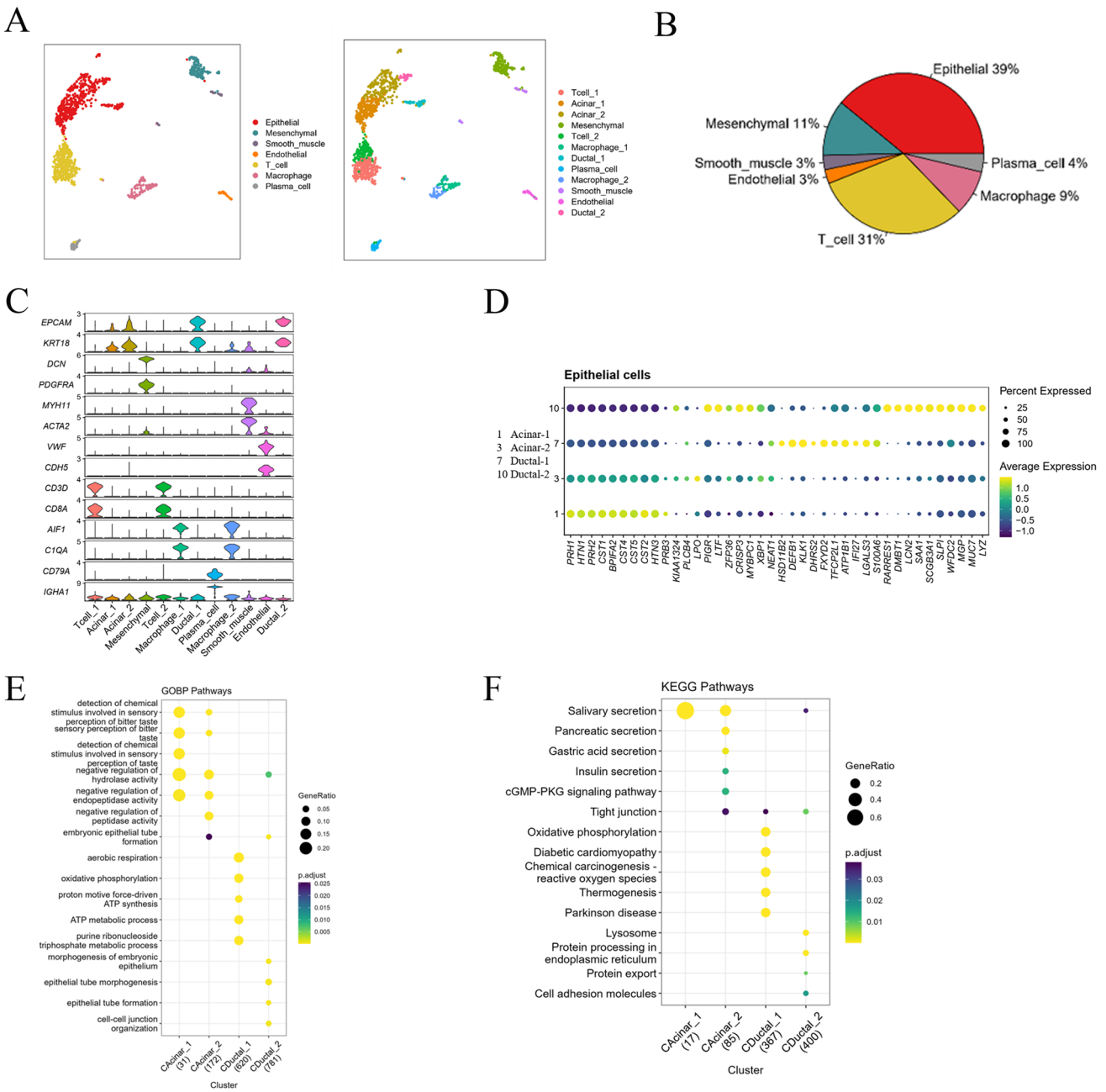



**Fig. 1** **A** The t-SNE plot for 1376 normal submandibular gland cells to visualize cell-type clusters based on the expression of known marker genes. **B** Proportion of each cell-type cluster. **C** Violin diagram showed the expression of specific genes in each cell-type cluster. **D** The characteristic genes of the four epithelial cell clusters. **E–F** Enrichment pathway of the four epithelial cell-type clusters based on GO (BP) and KEGG

characteristic genes (S1B). Here we analyzed the characteristic genes of four clusters of epithelial cells. *MGP*, *SLPI*, *LTF*, etc., are highly expressed in ductal-2, while *DEFB1*, *KLK1*, etc., are highly expressed in ductal-1. The characteristic genes of acinar-2 included *PDCD4*, *LPO*, etc. *CST2*, *PRH1*, etc., were the characteristic genes of acinar-1 (Fig. 1D). To further explore the function of the four clusters of epithelial cells, we conducted an enrichment analysis of cellular signal pathways based on GO biological processes (BP) and KEGG. The results indicated that acinar cells were primarily associated with "taste detection," "salivary secretion," "neutrophil activation," "ATP metabolic process," "neutrophil activation involved in immune response" "neutrophil mediated," and "cellular respiration" (Fig. 1E–F).

## Cluster analysis of normal and tumor tissues

We analyzed all the tissue cells together and obtained 6511 cells, including 1376 normal and 5135 tumor tissue cells (Fig. S2). Similarly, all cells were divided into seven different types and each type had its own characteristic gene signature (Fig. 2A–B). To further explore the difference between tumor and normal tissue, we conducted a comparative analysis. Figure 2C–D and Table S2 show that the amount of the epithelial cells in tumor tissues was significantly higher compared with that in normal tissue, but there were almost no T cells or plasma cells, which was significantly different from the latter. To further establish the heterogeneity of ACC, we conducted an in-depth analysis of tumor tissues. As is shown in Fig. 2E–F and Table S3, in tumor tissue, there were more epithelial cells (84.77%) and aneuploid cells as well as few T cells, plasma cells, mesenchymal cells, and macrophages.

## TME of salivary ACC

### Cluster analysis of nonimmune cells in tumor tissues

We extracted 5501 nonimmune cells from tumor and normal tissues for analysis. These nonimmune cells were divided into 13 clusters (Fig. 3A). The proportion of each type of cell in the two tissues was quite different (Fig. 3B–C and Table S4). The normal tissues were primarily composed of acinar cells, duct cells, interstitial cells, and a small number of endothelial and smooth muscle cells. The tumor tissues were comprised of four clusters of malignant epithelial cells (C1, C2, C5, and C6) and EMT cells. Acinar and duct cells in the tumor tissue disappeared completely. Next, we analyzed the expression of characteristic genes, highly expressed genes, and related signal pathways of the 4 cell types (Fig. 3D–E and S3). C2 expressed *NFIB* and *MYB* genes at the highest level, which may represent a characteristic cell cluster of ACC. In addition, C2 overexpressed several specific genes including *NTRK3*, *GAS6*, *ITGA2*, *MT2A*, *MT1E*, and *COL17A1*. The function of these genes is associated with high invasiveness and metastasis of tumor cells. C1 overexpressed the *EFNA5*, *SORS2*, and *SEMA3E* genes, C5 overexpressed *BAMBI, RFLNA*, *SIX3*, and *TTYH1* genes, and C6 overexpressed *TNFRSF12A*, *CXCL3*, *CXCL8*, *LRRC75A*, and *PLAUR*. The function of these genes is similar as they are closely associated with cellular signal transduction, growth, and development (Xie et al. 2022; Pils et al. 2010; Zhou et al. 2022; Li et al. 2013). In terms of function, the three cell types exhibited increased expression of the p53, Notch, and MYC signaling pathways, and their function was consistent with the characteristic genes. Another study reported a significant increase of NOTCH signaling in recurrent ACC, suggesting that treatment strategies could be formulated for targeting this signaling (Parikh et al. 2022).In addition, the C6 cluster was also associated with glycolysis. C7 was primarily associated with hypoxia, angiogenesis, epithelial mesenchymal transformation (EMT), as well as the MTORC1 and TGF-beta signaling pathways.

To verify the highly expressed genes in the 4 types of malignant cells, we selected an ACC sample from the right sublingual gland of a 57-year-old male with the cribriform pathological type and nerve invasion for immunofluorescence detection. The NTRK3, BAMBI, EFNA5, and TNFRSF12A were used for each cell type. Figure 3F shows that the staining intensity of NTRK3, BAMBI, EFNA5, and TNFRSF12A was increased in tumor and nerve tissues. The same result was confirmed in a 50-year-old female patient's samples with cribriform type and lymphatic invasion (Fig. S4 A) and this sequenced samples (Fig. S4 B), which were consistent with the results of single-cell sequencing.

To further explore the BP of the various cell types in tumor tissue, we performed a GO pathway enrichment analysis on these clusters (Fig. S5). The four clusters of malignant cells were primarily enriched for the terms "epidermis development," "translational initiation," "ossification," and "nuclear-transcribed mRNA catabolic process." Other clusters were mainly enriched with "extracellular matrix organization," "extracellular structure organization," and "ATP metabolic process."

### Immune infiltration analysis of TME

We divided 878 immune cells into 7 clusters (Fig. 4A). Compared with normal tissues, the immune signature of tumor tissues was reduced as a whole, which shows that CD8 + cytotoxic T and CD4 + T helper cells were significantly downregulated and IgA plasma cells responsible for regulating mucosal immunity completely disappeared (Fig. 4B and Table S5). The immune cells of ACC primarily included two clusters of macrophages. One cluster consisted

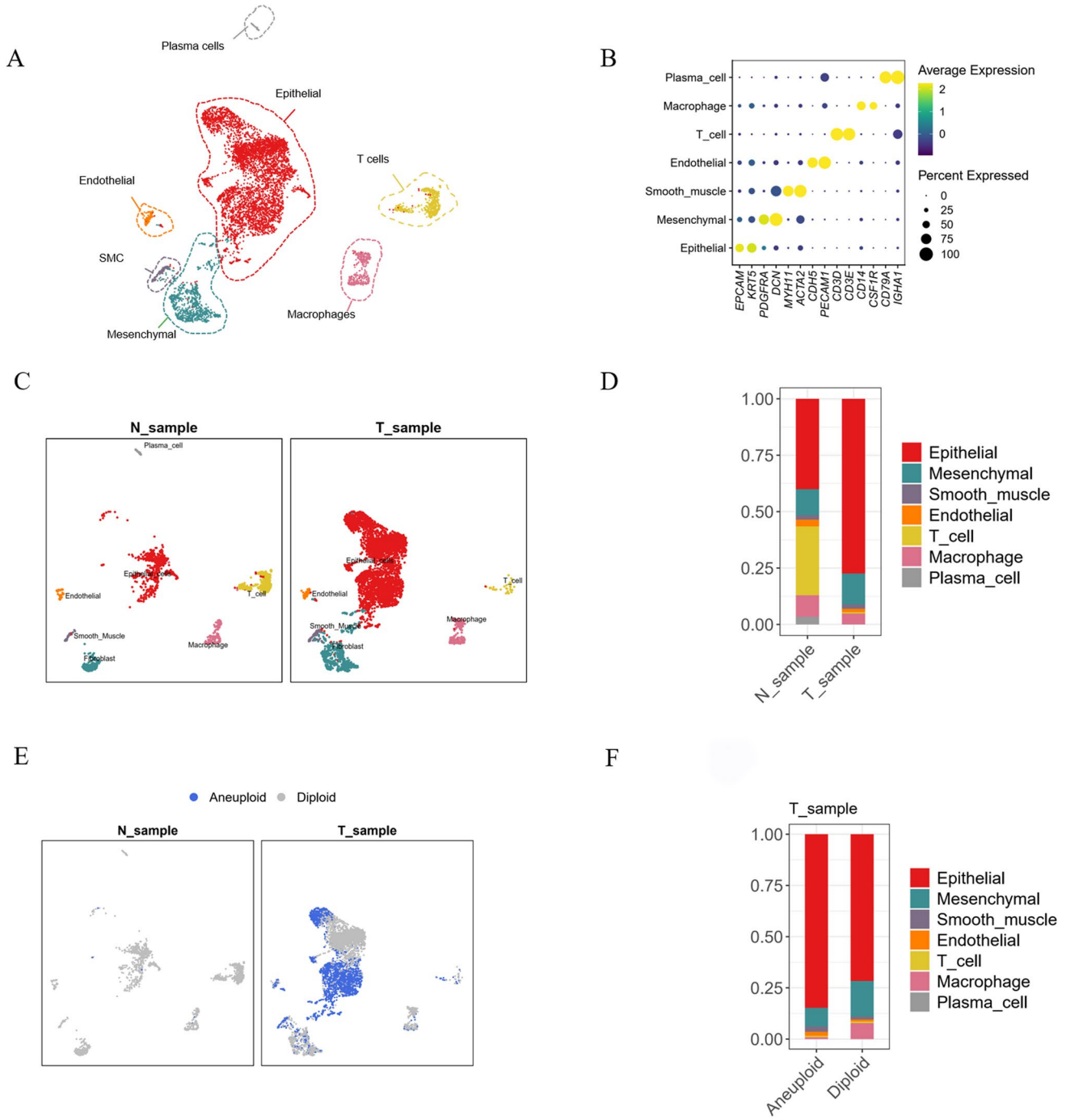



**Fig. 2** **A** The t-SNE plot of 6551 normal and tumor cells to visualize cell-type clusters based on the expression of known marker genes. **B** The characteristic genes of each cell-type cluster. **C–D** Proportion of each cell-type cluster in the two tissues. **E–F** Differences of aneuploid and diploid cells in the tumor tissue

of inflammatory macrophages (IL-1B) that also was present in normal tissues, whereas the other cluster was associated with the EMT, which was significantly higher compared with that in normal tissues. Next, we analyzed the expression of characteristic genes in these seven cell clusters. The heat map shows the top 10 specific genes of all immune cells (Fig. 4C). Cluster 1 and 2 expressed high levels of *CD83*, *CCL3*, *CXCL3*, *KRT14*, and *KRT117*.

Macrophages are an important immune cell type involved in defense from pathogens, chemotaxis, phagocytosis, secretion of lysozyme and other active substances, and antigen presentation. There were macrophages in ACC

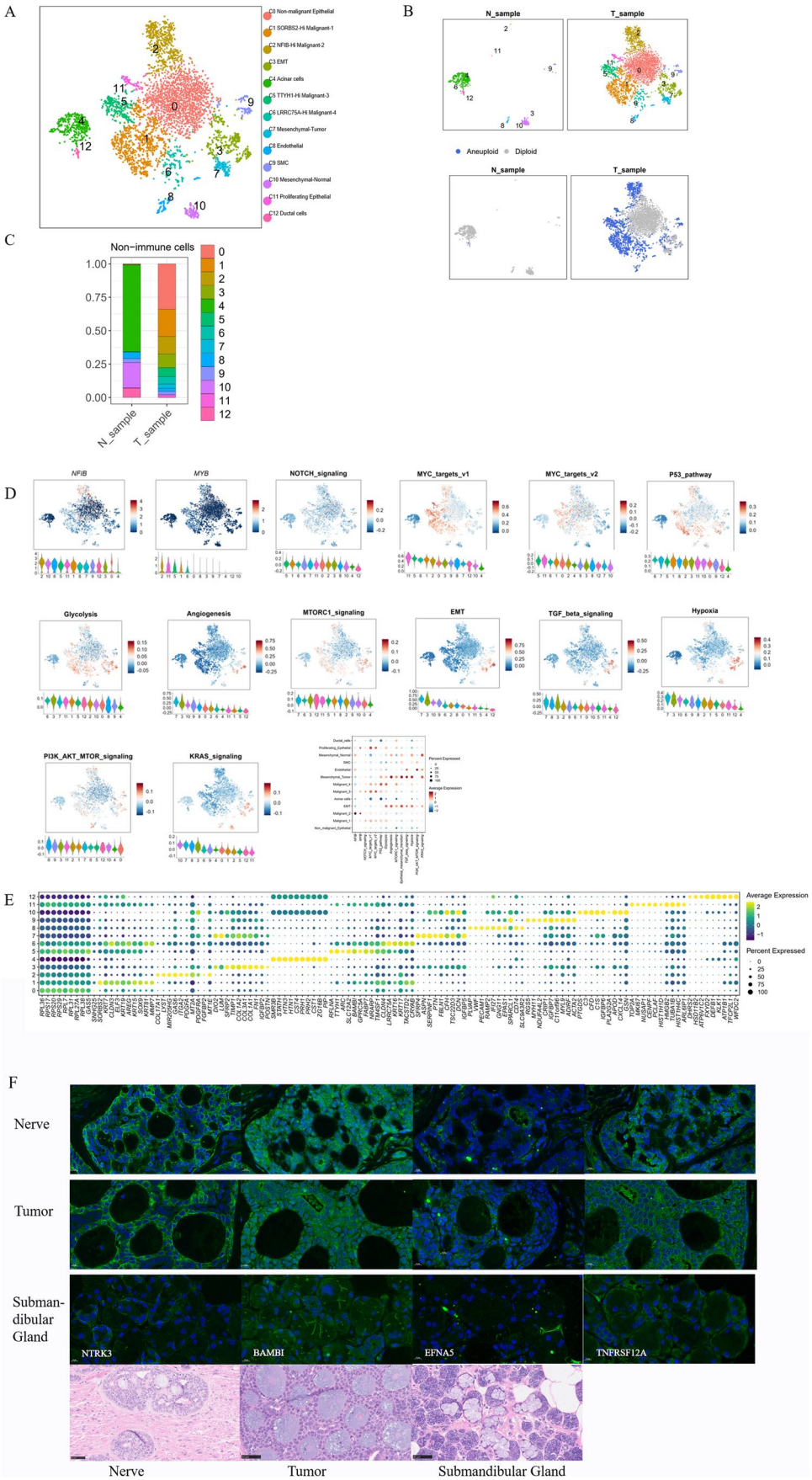



**Fig. 3** **A** The t-SNE plot of 5501 nonimmune cells from tumor and normal tissues to visualize cell-type clusters based on the expression of known marker genes. **B–C** Proportion of each cell-type cluster in the two tissues. **D** The highly expressed genes and related signaling pathways of each cell-type cluster. **E** The top 10 highly expressed genes in each cell-type cluster. **F** H&E (×40) and immunofluorescence (×100) of the ACC sample and normal submandibular gland tissue

and normal tissues, so we determined the differences by comparing the M1- and M2-specific gene expression profiles of macrophages in the two tissues (Fig. 4D). The M1 macrophages in tumor tissue highly expressed *BCL2A1*, *TNFRSF1B*, *FCGR2A*, *IL1B*, and *PSMA2*. M2 macrophages expressed *CCL3*, *CCL4*, *CD163*, *MSR1*, *CTSC*, *CTSD*, *FGL2*, *HEXB*, *MAF*, *MS4A6A*, and *SLC4A7*. To further explore the functional differences of macrophages in the two tissues, we conducted a GO (BP) enrichment analysis on these cell clusters (Fig. 4E). In normal tissues, macrophages primarily participate in antigen presentation through MHC-II and in the interaction between various cells. However, the function of macrophages in tumors was attributed primarily to "neutrophil activation," "neutrophil mediated immunity," "transcribed mRNA catabolic process, nonsense-mediated decay," and "SRP-dependent cotranslational protein targeting to membrane."

#### Intercellular interactions in TME

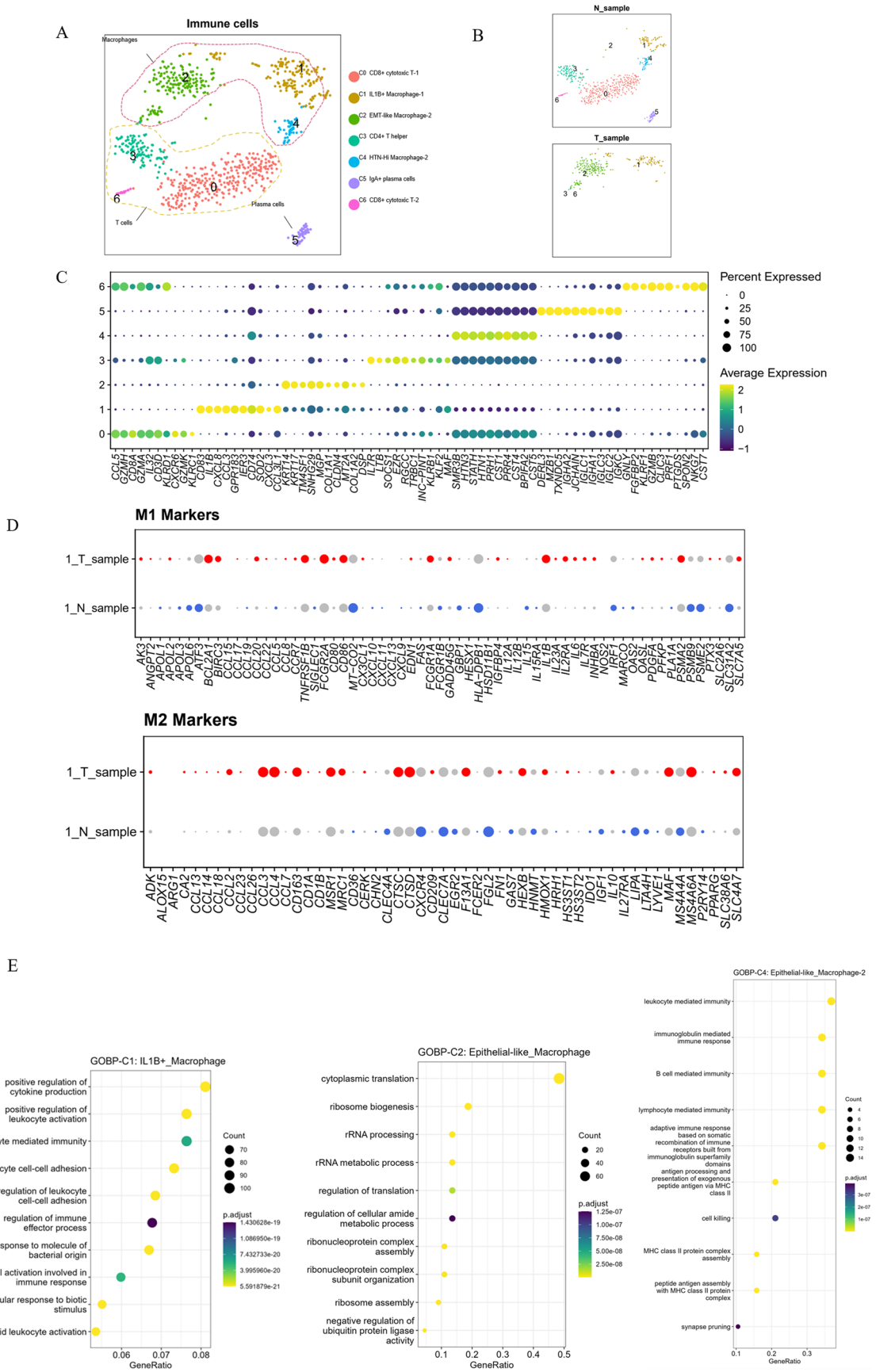
To further explore the interaction between different cell types, we constructed an intercellular communication network for all cells. Figure 5A–B shows the number and intensity of signal pathways between all types of cells in the two tissues. Compared with normal tissues, there were more connections between various cell types in tumors, especially between epithelial cells and other cell types. Interestingly, plasma cells had no contact with other cells in the tumor tissue.

Epithelial cells were the main component of ACC. Therefore, we focused on the difference in epithelial cell signaling in the two tissues (Fig. 5C–D). Epithelial cells in normal tissues highly expressed HLA, which primarily interacts with T cells through MHC-1 and with T cells and macrophages through MIF. Of these, HLA-CD8 was highly expressed and MIF-CD74 has obvious advantages. However, in tumor tissues, the HLA signaling pathway in epithelial cells was significantly downregulated. Epithelial cells primarily interact with macrophages and T cells through MIF, macrophages, T cells, and endothelial cells through APP, whereas T cells, endothelium, smooth muscle, and interstitial cells through collagen. The expression of APP-CD74 was significantly upregulated in macrophages and T cells in addition to MIF (CD74 + CXCR4) in T cells. In addition, compared with normal tissues, COL1A2, COL1A1, and MDK signaling pathways were also upregulated in T cells, smooth muscle cells, and mesenchymal cells. Figure S6A and Fig. S6B show the differences of receptor–ligand interactions and signal pathways of cells, respectively, in the two tissues.

## Discussion

ACC has been described as having a "lazy" growth mode, but a "long-term aggressive" behavior. Recurrence and distant metastasis are the primary causes of death. Based on its growth pattern and low survival rate, ACC is considered one of the most biologically destructive and unpredictable tumors of the head and neck (Spiro et al. 1974; Sequeiros Santiago et al. 2005). Currently, standard treatment includes radical surgical resection to ensure tumor-free margins and postoperative radiotherapy, however, there is no effective strategy for advanced or recurrent metastatic tumors. Therefore, it is of great significance to find effective strategies to manage this tumor. One important area of focus is the changes that occur in the TME and the heterogeneity of ACC. We identified the various cell types in ACC and determined the differences in the microenvironment between tumor and normal tissues.

The proportion of epithelial cells in the ACC tissue increased significantly, whereas the number of immune cells decreased markedly, such as T cells. Only a few plasma cells and 0.92% of aneuploid macrophages were observed, which was consistent with a previous report (Mosconi et al. 2019). The microenvironment was considered type IV (immune tolerant), which indicates that immunotherapy is not suitable for this tumor. The C2 cluster highly expressed *NFIB* and *MYB*, which are characteristic ACC genes. In addition, this cell cluster overexpressed multiple genes associated with tumor invasion and metastasis, especially NTRK3, which encodes a member of the neurotrophic tyrosine receptor kinase family. This suggests that the function of these cells may be related to neural pathways, which is consistent with the clinical features of the growth and nerve infiltration characteristics of ACC (Ivanov et al. 2013). BAMBI is a transmembrane glycoprotein related to the type I receptors of the transforming growth factor-beta (TGF-β) family and involved in the regulation of tumor cell proliferation and metastasis via Wnt-β-catenin pathway and the pathways involved in the regulation of the cell cycle (Zhou et al. 2013; Amerongen et al. 2008; Massagué 2008). EFNA5 is a member of the ephrin gene family and related to tumor invasion and progression (Li et al. 2020; Ebrahim et al. 2021; Ricci et al. 2020). TNFRSF12A, also known as CD266, FN14, and TWEAKR, is a member of the tumor necrosis factor receptor superfamily. It was reported that TNFRSF12A played an important role in the development, progress, and drug resistance of various types of cancer (Wang et al. 2017; Xia et al. 2021; Yao et al. 2020). Compared with normal tissue, the staining intensity of the four genes were increased in the tumor tissues of invaded nerve, lymph node, and tumor body. The correlation between these four genes and the biological behavior of ACC should be further studied.


A
Immune cells
Macrophages
T cells
Plasma cells
C0 CD8+ cytotoxic T-1
C1 IL1B+ Macrophage-1
C2 EMT-like Macrophage-2
C3 CD4+ T helper
C4 HTN-HI Macrophage-2
C5 IgA+ plasma cells
C6 CD8+ cytotoxic T-2
B
N_sample
T_sample
C
Percent Expressed
Average Expression
D
M1 Markers
1_T_sample
1_N_sample
M2 Markers
E
GOBP-C1: IL1B+_Macrophage
positive regulation of cytokine production
positive regulation of leukocyte activation
leukocyte mediated immunity
leukocyte cell-cell adhesion
regulation of leukocyte cell-cell adhesion
regulation of immune effector process
response to molecule of bacterial origin
cell activation involved in immune response
cellular response to biotic stimulus
myeloid leukocyte activation
GeneRatio
GOBP-C2: Epithelial-like_Macrophage
cytoplasmic translation
ribosome biogenesis
rRNA processing
rRNA metabolic process
regulation of translation
regulation of cellular amide metabolic process
ribonucleoprotein complex assembly
ribonucleoprotein complex subunit organization
ribosome assembly
negative regulation of ubiquitin protein ligase activity
GeneRatio
GOBP-C4: Epithelial-like_Macrophage-2
leukocyte mediated immunity
immunoglobulin mediated immune response
B cell mediated immunity
lymphocyte mediated immunity
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains
antigen processing and presentation of exogenous peptide antigen via MHC class II
cell killing
MHC class II protein complex assembly
peptide antigen assembly with MHC class II protein complex
synapse pruning
GeneRatio

◂**Fig. 4** **A** The t-SNE plot of 878 immune cells from tumor and normal tissues to visualize cell-type clusters based on the expression of known marker genes. **B** Proportion of each cell-type cluster in the two tissues. **C** The top 10 highly expressed genes of each cell-type cluster. **D** Difference in highly expressed genes in M1 and M2 macrophages in the two tissues. **E** Enrichment pathway of the macrophages in the two tissues based on GO (BP)

CD74, also known as HLA-DR antigens-associated invariant chain, is primarily used as a chaperone of MHC class II molecules and is highly expressed in immune cells, such as dendritic cells, monocytes/macrophages, and B cells. It also participates in the transport of other non-MHC-II proteins and is a high affinity receptor for MIF, D-DT/MIF-2, CXCR4, and bacterial proteins on the cell membrane (Su et al. 2017). Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine secreted by various immune cells including monocytes, macrophages, dendritic cells, granulocytes, and mast cells. The combination of MIF and CD74 is essential for cell signal transduction, but it is not enough to trigger intracellular signal transduction. They need to combine with CD44 or CXCR4 to form a coreceptor protein to activate downstream signal pathways, such as the PI3K-AKT, ERK/MAP, and NF-kB signaling cascades (Noe and Mitchell 2020), to promote tumor development and progression (Koh and Kim 2020). MIF is highly expressed in a variety of tumors and is a potential prognostic factor associated with poor survival (Osipyan et al. 2021). Amyloid precursor protein (APP) is a type I transmembrane

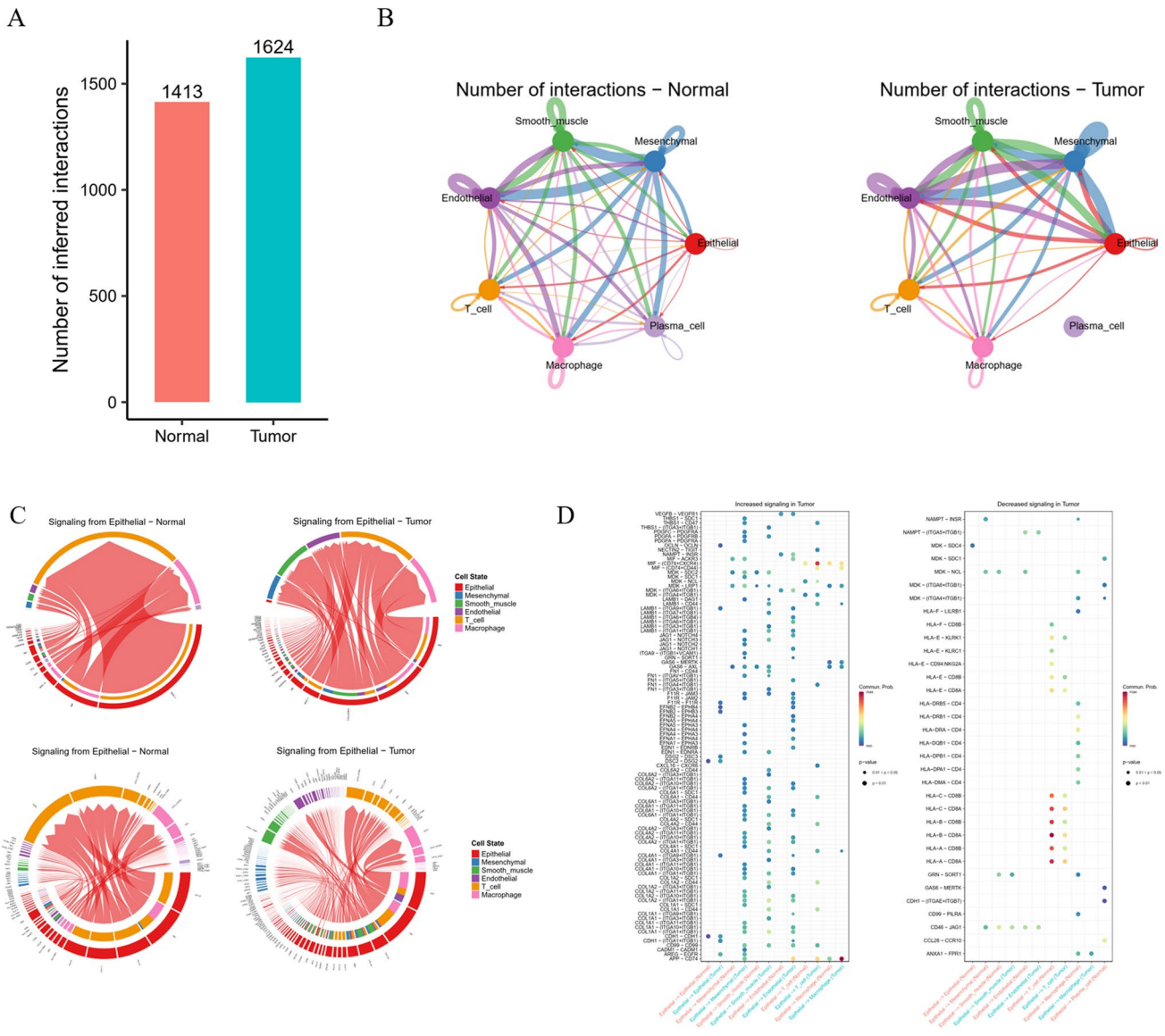



**Fig. 5** **A**–**B** The number and intensity of signaling pathways between all cell-type clusters in the two tissues. **C**–**D** Difference in signal transduction between epithelial cells and other cells in the tumor microenvironment

protein, which is widely expressed in the mammalian brain. It is processed through a series of proteolytic steps and is a widely studied protein in Alzheimer's disease (Zheng and Koo 2011). In recent years, APP and its cleaved forms have been implicated in the development of metabolic diseases, particularly sAPP and AICD, which are associated with the progression of various cancers, including colon cancer, pancreatic cancer, oral squamous cell carcinoma, non-small cell lung cancer, and advanced breast cancer. Although studies have confirmed that the increase in expression was closely associated with the progression and invasion of cancer cells, the specific mechanism remains unclear (Meng et al. 2001; Ko et al. 2004; Hansel et al. 2003; Lim et al. 2014; Sobol et al. 2015). In the present study, we found that the epithelial cells in ACC interact with T cells, macrophages, and other cells through APP, MIF, and collagen. In the TME, APP, MIF, COL1A2, COL1A1, and MDK pathways were significantly upregulated with respect to the communication of various cell clusters. Our results were consistent with another study, MIF-CD74 and APP-CD74 notably upregulated and related to tumor antigen presentation and intercellular communication in TME, which may represent a target for future studies.

Although we have made some discoveries of ACC, there are still some limitations to this study. First, more tumor samples with various pathological subtypes are needed for single-cell sequencing, including the cribriform, tubular, and solid type. Secondly, the markers identified in the four clusters of malignant cells require validation in a larger number of samples. Finally, factors associated with the recurrence and metastasis of ACC need to be analyzed. Studying the specific genes and their function in malignant epithelial cells will enable us to identify mechanisms of recurrence/metastasis in ACC.

In conclusion, we conducted a scRNAseq analysis of ACC. This study revealed the heterogeneity and microenvironment associated with this tumor, which provides insight into its composition, function, and interrelationship among the various cell types. We identified four clusters of malignant epithelial cells in ACC and analyzed their genetic characteristics and putative functions. MIF-CD74 and APP-CD74, particularly the latter, represent potential targets for the diagnosis and treatment of ACC.

## Conclusion

We explored the heterogeneity and the TME of an ACC sample from a single cell perspective, including cell characteristics, immune infiltration, and communication networks, which provide further insight into ACC and may lead to new treatment strategies.

**Abbreviations** *ACC*: Adenoid cystic carcinoma; *SACC*: Adenoid cystic carcinoma of the salivary gland; *TME*: Tumor microenvironment; *EMT*: Epithelial mesenchymal transition; *GO*: Gene Ontology; *KEGG*: Kyoto Encyclopedia of Genes and Genomes; *APP*: Amyloid precursor protein; *BP*: Biological processes; *MIF*: Migration inhibitory factor; *NSCLC*: Non-small cell lung cancer

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1007/s10142-023-01082-4.

**Author contribution** Jie Zhang contributed to the concept and designed the research. Quality control of data and algorithms was performed by Pu-gen An and Wen-Jie Wu. Data analysis and statistical analysis were performed by Pu-gen An, Wen-Jie Wu, and Yu-Fang Tang. Pu-gen An and Wen-Jie Wu commented on the manuscript preparation and editing. Jie Zhang performed the manuscript review critically and approved the final version.

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**Data availability** The datasets used and analyzed during the current study are available from the National Library of Medicine (NCBI), BioProject accession number PRJNA900693, BioSample accession number SAMN31699269, SRA accession number SRP407714 and online at https://dataview.ncbi.nlm.nih.gov/object/PRJNA900693?reviewer=em240tlu64753jaqf3m4aqhind.

## Declarations

**Ethical approval** We declare that all methods were carried out in accordance with the Declaration of Helsinki and all experimental protocols were approved by the Ethics Committee of Peking University School and Hospital of Stomatology (IRB number: PKUSSIRB-202165081). Written informed consent was obtained from all participants in the study. The privacy rights of the participants will be observed.

**Competing interests** The authors declare no competing interests.

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