Heterogeneity and microenvironment in temporal bone squamous cell carcinoma based on single-cell ribonucleic acid sequencing

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ABSTRACT

Temporal bone squamous cell carcinoma (TBSCC) is the most common subtype of the temporal bone malignancies. Due to its low incidence, high rates of misdiagnosis, and challenges in conducting large-scale studies and clinical trials, its mechanisms of occurrence and progression remain unclear, and both diagnostic and therapeutic biomarkers are lacking. These challenges largely depend on our understanding of the tumor heterogeneity and microenvironment of TBSCC. Since single-cell RNA sequencing (scRNA-seq) is an effective tool for studying tumor heterogeneity, we here first reported the scRNA-seq data of TBSCC in this study. We collected and sequenced a total of 113,344 cells from 9 samples (i.e., 5 cancer and 4 paracancerous tissues) of 6 TBSCC patients. We found the proportion of S-stage cancer cells was positively correlated with the risk of tumor infiltration, and the pseudo-time trajectory of cancer cells closely corresponded with TBSCC differentiation level. We also detected higher communication intensity between some immune cell pairs in cancer tissues than in paracancerous tissues. Additionally, some cancer-related genes are identified as potential diagnostic markers of TBSCC. This study is the first to unveil the complex heterogeneity and tumor microenvironment characteristics of TBSCC using scRNA-seq technology, offering new avenues for diagnosing, classifying, and treating this rare carcinoma.

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INTRODUCTION

Malignant temporal bone tumors are rare, with squamous cell carcinoma (TBSCC) being the predominant form. Diagnosis is challenging due to non-specific symptoms like otalgia and hearing loss, often leading to delayed detection. The mainstay of treatment is radical resection, yet outcomes remain poor due to high local recurrence rates and a lack of effective salvage therapies or specific biomarkers for personalized treatment.

The biological complexity of TBSCC is driven by significant tumor heterogeneity and a dynamic tumor microenvironment (TME), which are critical in tumor progression, immune evasion, and therapy resistance. To dissect this complexity, our study employed single-cell RNA sequencing (scRNA-seq) to analyze the cellular and molecular landscape of TBSCC and matched paracancerous tissues at an unprecedented resolution.

This deep characterization aims to map the diverse cell populations, their gene expression profiles, and interactions within the TME. We seek to uncover the fundamental mechanisms of TBSCC pathogenesis, with the goal of informing the development of novel diagnostic tools, prognostic biomarkers, and more effective, targeted therapeutic strategies for this challenging disease.

METHODS AND MATERIALS

Following ethical approval, five TBSCC tumor and four adjacent normal tissue samples were collected from six patients. Specimens were processed for single-cell RNA sequencing (scRNA-seq) and multiplex immunohistochemistry (mIHC).

For scRNA-seq, cells from the BD Rhapsody™ platform underwent quality control, retaining those with gene counts between 200-5000 and <25% mitochondrial content. Data was processed using Seurat in R for normalization, dimensionality reduction, and clustering. Cell types were annotated with SingleR. Cancer cells were identified via inferCNV based on copy number variation (CNV) scores (≥0.0016) and correlation coefficients (≥0.4). Differential gene expression and Gene Ontology enrichment analyses were performed. Cell cycle analysis, pseudo-time trajectory inference, and cell-cell communication analysis were conducted using respective R packages.

For mIHC, formalin-fixed paraffin-embedded sections were stained with primary antibodies, HRP-conjugated secondaries, and tyramide signal amplification fluorophores. Images were acquired using a PANNORAMIC MIDI II slide scanner.

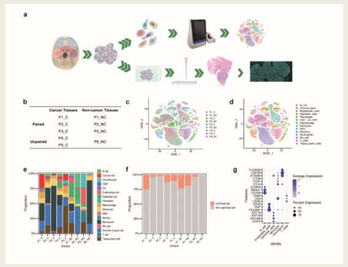

Figure 1. TBSCC single-cell atlas.

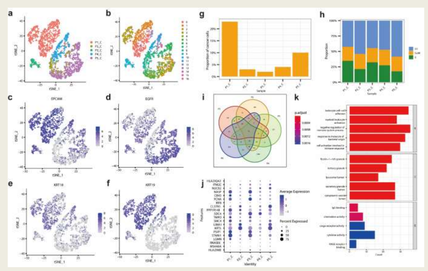

Figure 2. Heterogeneity analysis on TBSCC cells.

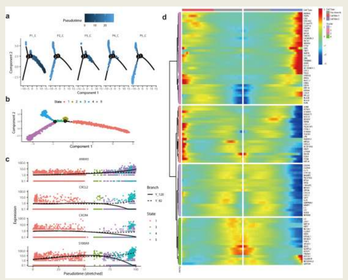

Figure 3. Pseudo-time analysis on TBSCC cells.

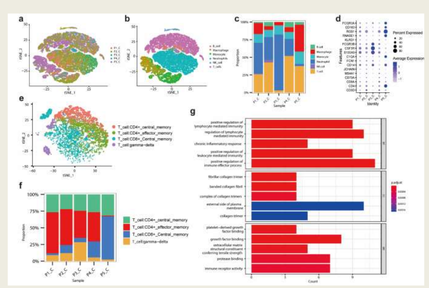

Figure 4. Tumor microenvironment of TBSCC.

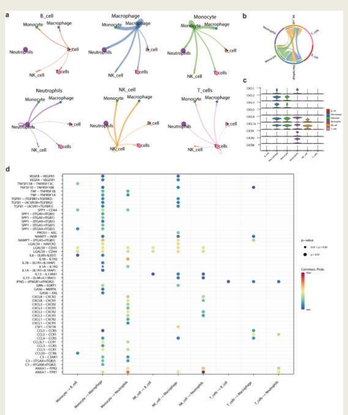

Figure. 5 Cell communication networks.

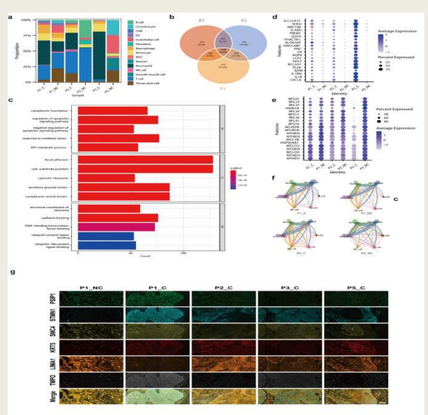

Figure. 6 Differential analysis of homologous.

RESULTS

ScRNA-seq of six TBSCC patients revealed a complex tumor ecosystem. Epithelial cells exhibited significant heterogeneity, while immune and stromal cells clustered by type. InferCNV analysis identified malignant cells with widespread copy number variations, confirming inter- and intra-tumoral heterogeneity. Pseudotime trajectory analysis revealed two distinct differentiation fates in TBSCC cells, with key genes (ANXA5, CXCL2, CXCR4, S100A9) showing time-dependent expression patterns.

The immune microenvironment varied substantially between patients. Sample P3_C showed neutrophil predominance (79.24%) with minimal T cells (2.93%), while P5_C was macrophage-rich (38.11%). Cell-cell communication analysis identified strong interactions via ANXA1-FPR1 and NAMPT-(ITGA5+ITGB1) pathways. TGF-β signaling was particularly active among macrophages, monocytes and NK cells.

Comparison of matched tumor-paracancerous tissues identified 1308 consistently upregulated and 454 downregulated genes. Multiplex immunohistochemistry validated protein-level expression of candidate markers (STMN1, PSIP1, KRT5) across patient samples, confirming the scRNA-seq findings and highlighting potential diagnostic biomarkers.

DISCUSSION

Despite its rarity, temporal bone squamous cell carcinoma (TBSCC) presents significant diagnostic and therapeutic challenges due to its high heterogeneity. This study employed single-cell RNA sequencing on five tumor and four matched paracancerous tissues from six patients to dissect this complexity.

We constructed a detailed cellular atlas, revealing substantial inter- and intra-tumoral heterogeneity. Epithelial cells showed sample-specific clustering patterns, while immune and stromal cells grouped by cell type. Pseudotime analysis identified two distinct differentiation trajectories in cancer cells, with varying cell cycle distributions suggesting different metastatic potentials. The tumor microenvironment exhibited patient-specific immune compositions, with some cases showing neutrophil predominance and others macrophage richness. Cell-cell communication analysis revealed strong interactions via specific pathways including ANXA1-FPR1 and TGF-β signaling.

Comparative analysis of paired tissues identified consistently dysregulated genes, validated by multiplex immunohistochemistry. These findings provide insights into TBSCC pathogenesis and highlight potential biomarkers for diagnosis and treatment. The heterogeneity observed underscores the need for personalized therapeutic approaches and suggests single-cell profiling could inform clinical decision-making for this complex malignancy.

CONCLUSIONS

A systematic scRNA-seq analysis of the heterogeneity and microenvironment of TBSCC was conducted. This study uncovered multi-dimensional heterogeneity and key characteristics of the TBSCC microenvironment, along with DEGs pertinent to the diagnosis, classification, differentiation, and infiltration of TBSCC. These findings enhance our understanding of TBSCC and could significantly impact its diagnosis and treatment approaches.

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