SLCO1B3: A Prognostic Marker and Immune Modulator in Oral Squamous Cell Carcinoma

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Abstract. While clinical management of oral squamous cell carcinoma (OSCC) has seen substantial progress, its incidence and mortality rates have shown minimal improvement over the past three decades. Recent studies highlight the therapeutic potential of solute carrier organic anion transporter family member 1B3 (SLCO1B3) in head and neck squamous cell carcinoma, yet its precise mechanistic role in OSCC remains elusive. Through pan-cancer expression profiling, we identified marked heterogeneity in SLCO1B3 expression across tumor types, with marked upregulation specifically observed in OSCC tissues. Systematic analysis of clinical datasets revealed a significant inverse correlation between SLCO1B3 expression levels and critical prognostic indicators, including overall patient survival. Functional enrichment analysis further demonstrated SLCO1B3's robust association with immune infiltration signatures, suggesting its involvement in shaping the tumor immune microenvironment. By integrating ESTIMATE microenvironment scoring and single-sample gene set enrichment analysis (ssGSEA), we uncovered SLCO1B3-mediated suppression of B-cell populations—particularly naive B cells—revealing a novel mechanism of immune evasion. These findings not only establish SLCO1B3 as a prognostic biomarker for OSCC but also provide mechanistic insights for developing immune microenvironment-targeted therapies.

Keywords: SLCO1B3; Oral Squamous Cell Carcinoma; A Prognostic Marker and Immune Modulator.

1. Introduction

Oral cancer constitutes a major global health burden as the eighth most prevalent malignancy, accounting for over 377,000 new cases in 2020, of which >90% were histologically classified as oral squamous cell carcinoma (OSCC) [1]. Despite escalating annual incidence rates, conventional therapeutic regimens—primarily surgical resection with adjuvant radiotherapy/chemotherapy—yield modest prognostic improvements. The dual limitations of poor metastatic targeting and chemotherapy-induced nonspecific cytotoxicity underscore the urgency for novel treatment paradigms [2]. While molecularly targeted therapies represent promising alternatives, critical barriers including biomarker reliability, therapeutic resistance, and target identification remain unresolved [3]. This therapeutic impasse necessitates precise target discovery within OSCC's distinctive tumor microenvironment (TME), a prerequisite for advancing treatment efficacy [4].

Originally characterized as a hepatic basolateral membrane transporter (chromosome 12p12-31.7-37.2), SLCO1B3 mediates cellular uptake of endogenous/exogenous substrates for metabolic clearance. Emerging oncological research has paradoxically identified its aberrant overexpression across multiple malignancies, though its functional dichotomy in OSCC remains unexplored [5].

The dynamic reciprocity between neoplasms and their stromal ecosystem—comprising immune infiltrates, fibroblasts, and remodeled extracellular matrices—serves as a critical driver of oncogenesis [6,7]. Tumor cells orchestrate microenvironmental reprogramming through angiogenic induction, immune tolerance mechanisms, and paracrine signaling, while reciprocally, TME constituents modulate neoplastic evolution [8]. This bidirectional crosstalk governs metastatic dissemination and therapeutic resistance through autocrine/paracrine regulatory loops [9]. Consequently, comprehensive TME deconvolution coupled with molecular target identification emerges as a strategic imperative for optimizing immunotherapeutic interventions [10]. Precision targeting of TME components may potentially circumvent systemic toxicity while enhancing antitumor specificity, thereby addressing a fundamental constraint of conventional therapies [11].

Our investigation elucidates SLCO1B3's multifaceted role in OSCC pathobiology. Pan-cancer transcriptomic profiling revealed tumor-type-specific SLCO1B3 expression patterns, with pronounced enrichment in OSCC specimens. Clinico-genomic integration demonstrated significant inverse correlations between SLCO1B3 levels and survival endpoints. Mechanistically, multimodal analyses—incorporating ESTIMATE and ssGSEA algorithms—uncovered its immunosuppressive function via selective depletion of B-cell lineages, particularly naive B-cell subsets. These findings collectively nominate SLCO1B3 as both a prognostic indicator and a therapeutic node for TME-focused OSCC management.

2. Materials and Methods

2.1 Data Acquisition and Gene Expression Profilin

RNA-sequencing data (STAR-aligned, TPM-normalized) encompassing 33 malignancies were sourced from TCGA (https://portal.gdc.cancer.gov; accessed 12 May 2023), with matched normal tissue profiles obtained from GTEx. All analyses were executed in R v4.2.1 utilizing core statistical packages (stats v4.2.1; car v3.1-0) for hypothesis testing and ggplot2 v3.3.6 for visualization. Comparative analyses between groups employed Wilcoxon rank-sum tests, with significance thresholds defined as: ns ($p \geq 0.05$), * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

2.2 Survival Analysis

Kaplan-Meier methodology assessed SLCO1B3's prognostic value across three endpoints: OS, PFI, and DSS. The survival v3.3.1 package facilitated proportional hazards modeling, with survminer/ggplot2 generating graphical outputs. Log-rank tests determined curve divergence significance.

2.3 Clinical Relevance of SLCO1B3 in OSCC

To explore the clinical utility of SLCO1B3 in oral squamous cell carcinoma (OSCC), analyses including receiver operating characteristic (ROC) curves, risk scoring, calibration plots, and nomogram models were performed. The pROC package (v1.18.0) was used for ROC analysis, excluding samples lacking clinical annotation. Cox regression and proportional hazards analyses were conducted using the survival package (v3.3.1), while the rms package (v6.3-0) facilitated calibration and nomogram construction. Time-dependent ROC analysis was carried out using timeROC (v0.4). All results were visualized using ggplot2 (v3.3.6).

2.4 Co-Expression and Functional Enrichment Analysis in OSCC

TCGA OSCC data were stratified by SLCO1B3 expression (high vs. low) to identify co-expressed genes. Raw count matrices were processed via DESeq2 (v1.36.0) for differential expression analysis. Heatmaps (ggplot2) and chord diagrams (circlize v0.4.1) illustrated correlations between SLCO1B3 and its top five positively/negatively associated genes. Functional enrichment (KEGG, GO) and Gene Set Enrichment Analysis (GSEA) were performed using clusterProfiler (v4.4.4), with GO term Z-scores calculated via GOplot (v1.0.2).

2.5 Immune Checkpoint and Pathway Analysis

Pan-cancer expression data (TCGA, TARGET, GTEx; N=19,131 samples) were retrieved from the UCSC Xena platform. SLCO1B3 (ENSG00000111700) and 60 immunomodulatory genes (24 inhibitory, 36 stimulatory; reference DOI: 10.1016/j.immuni.2018.03.023) were analyzed. Log2-transformed values [$\log2(x + 0.001)$] were subjected to Pearson correlation analysis between SLCO1B3 and immune pathway markers, restricted to primary tumor samples.

2.6 Immune Cell Infiltration Assessment

Immune infiltration levels were quantified using two approaches: (1) single-sample GSEA (GSVA v1.46.0) for 24 cell types (Bindea et al., 2013) and (2) CIBERSORTx for 22 cell subsets. Spearman correlations linked SLCO1B3 expression to immune cell abundance, while Wilcoxon rank-sum tests compared infiltration between high/low SLCO1B3 groups. Results were visualized with ggplot2 (v3.3.6).

2.7 Statistical Methods

Continuous data are reported as mean ± SD (≥3 replicates). Unpaired t-tests and one-way ANOVA compared two or multiple groups, respectively. Survival analyses employed Kaplan-Meier curves with log-rank testing. A significance threshold of $p < 0.05$ was applied throughout.

3. Results

3.1 Differential Expression of SLCO1B3 in Pan-Cancer and Oral Squamous Cell Carcinoma (OSCC)

An analysis of SLCO1B3 expression across 33 tumor types and their matched normal tissues revealed significant differential expression. Notably, SLCO1B3 was markedly upregulated in liver hepatocellular carcinoma (LIHC), head and neck squamous cell carcinoma (HNSC), and colon adenocarcinoma (COAD), with similar trends observed in paired tumor-normal samples (Figure 1A, B). Volcano plot results further confirmed that SLCO1B3 expression was specifically elevated in OSCC samples (Figure 1C). Moreover, SLCO1B3 expression was significantly higher in OSCC compared to normal tissues in TCGA, as well as in paired sample comparisons (Figure 1D, E).

3.2 Prognostic and Clinical Implications of SLCO1B3 in OSCC

Kaplan-Meier analysis revealed a robust association between elevated SLCO1B3 expression and adverse clinical outcomes in OSCC, demonstrating significantly reduced overall survival (OS; log-rank $P < 0.001$), progression-free interval (PFI; $P = 0.002$), and disease-specific survival (DSS; $P = 0.004$) (Figures 2A–C). Risk stratification models further corroborated SLCO1B3's prognostic value, with high-expression cohorts exhibiting markedly worse survival trajectories (Figure 2D). Diagnostic evaluation via ROC curve analysis yielded an AUC of 0.832 (95% CI: 0.857–0.929), confirming SLCO1B3's discriminative capacity for OSCC detection (Figure 2E). Time-dependent ROC analysis highlighted sustained predictive accuracy, particularly for 1-year (AUC = 0.801) and 2-year (AUC = 0.784) survival prognostication (Figure 2F).

Calibration plots demonstrated strong concordance between predicted and observed 1-year survival probabilities (Brier score = 0.12), reinforcing SLCO1B3's utility as an independent prognostic biomarker (Figures 2G–H). Univariate Cox regression identified SLCO1B3 overexpression as significantly correlated with advanced T-stage (T3: HR = 3.980, 95% CI: 1.677–9.447, $P = 0.002$; T4: HR = 3.717, 95% CI: 1.592–8.676, $P = 0.002$), nodal metastasis (N2/N3: HR = 2.253, 95% CI: 1.523–3.331, $P < 0.001$), and suboptimal therapeutic response (complete response [CR]: HR = 0.172, 95% CI: 0.112–0.265, $P < 0.001$). Multivariate analysis retained nodal stage N2/N3 (HR = 1.781, 95% CI: 1.123–2.826, $P = 0.014$) and CR status (HR = 0.258, 95% CI: 0.159–0.417, $P < 0.001$) as SLCO1B3-associated prognostic determinants (Table 1).

3.3 SLCO1B3-Associated Transcriptomic Alterations in OSCC

Comparative analysis (SLCO1B3 high vs. low groups) identified 817 differentially expressed genes (DEGs; 326 upregulated, 491 downregulated; adj. $P<0.05$, $|\log2FC|>1$), including prominently downregulated ZPBP2, GBX1, and AC0071159.1, and upregulated CALB1, SLCO1B3-SLCO1B7, and MAGEB1 (Figures 3A–C). Functional annotation revealed DEG enrichment in muscle-related processes (contraction, myofibril assembly) and structural components (GO terms), alongside

pathways implicating complement/coagulation cascades (KEGG:hsa04610), xenobiotic metabolism (hsa00980), and DNA adduct formation (hsa05204). GSEA further highlighted activation of complement signaling (NES=2.34), extracellular matrix remodeling (NES=2.11), and lipid metabolism (NES=1.98), suggesting SLCO1B3's dual role in immune-metabolic dysregulation.

3.4 SLCO1B3 Interactions with Immune Checkpoint Machinery

Pan-cancer analysis revealed broad positive correlations between SLCO1B3 expression and immunomodulatory genes (Figures 4–5). In OSCC, significant co-expression was observed with checkpoint markers CD274 (ρ=0.32, P=0.004) and HAVCR2 (ρ=0.28, P=0.011) (Figures 4A,5A), though validation across tumor types remains warranted.

3.5 SLCO1B3-Mediated Immune Microenvironment Remodeling

Pan-cancer immune infiltration analysis demonstrated SLCO1B3-associated depletion of key effector populations: T effector memory cells (Tem: ρ=−0.190), Th17 cells (ρ=−0.234), and regulatory T cells (Tregs: ρ=−0.171) (Figures 6B–C). Notably, naïve B-cell infiltration showed significant negative correlation (ρ=−0.200, P=0.007) and was markedly reduced in SLCO1B3-high cohorts (P<0.001; Figures 6D–H), suggesting its role in fostering an immunosuppressive niche.

4. Discussion

Oral squamous cell carcinoma (OSCC), representing nearly 90% of oral malignancies, severely compromises critical functions including speech, deglutition, and aesthetic integrity[12]. Projections indicate a 40% rise in OSCC incidence and mortality by 2040, reflecting its escalating global health burden [12]. Despite therapeutic advancements such as PD-L1 inhibitors, survival outcomes remain suboptimal—5-year overall survival (OS) rates decline from 70–90% in early-stage disease to ~50% in advanced stages [13,14], underscoring the imperative for novel prognostic biomarkers and targeted interventions.

Solute carrier organic anion transporter 1B3 (SLCO1B3), a hepatocyte basolateral membrane transporter, facilitates the uptake of endogenous substrates and chemotherapeutic agents (e.g., taxanes, camptothecins) [15]. While implicated in therapy resistance across malignancies—including androgen deprivation resistance in breast cancer and drug efflux modulation in colorectal/liver cancers [16-17]—its role in OSCC pathogenesis remains unexplored.

Our pan-cancer analysis integrating TCGA and GTEx data identified SLCO1B3 overexpression across multiple tumors, with pronounced upregulation in OSCC. Elevated SLCO1B3 expression correlated with adverse prognosis via Kaplan-Meier survival curves, Cox regression, and risk modeling. Functional enrichment implicated immune dysregulation as a central mechanism, positioning SLCO1B3 as both a prognostic biomarker and potential therapeutic target.

The tumor immune microenvironment (TME), a dynamic network of immune infiltrates, stromal cells, and vasculature, critically influences immunotherapy responsiveness. Tumor-driven TME remodeling facilitates angiogenesis, immune evasion, and chemoresistance [18]. For instance, lipid metabolism-mediated Treg activation in gastric cancer [19] and TTC22-induced dendritic cell immaturity in pancreatic cancer [20] exemplify mechanisms of immune suppression.

B cells, traditionally associated with humoral immunity, exhibit context-dependent roles in tumor biology. While CXCR4+ B cell infiltration correlates with improved gastric cancer outcomes [22] and CCL19-driven tertiary lymphoid structures enhance antitumor immunity [23], their function in OSCC remains poorly characterized [24].

We propose that SLCO1B3 fosters an immunosuppressive TME in OSCC by attenuating naïve B cell infiltration and antigen presentation. Pan-cancer analyses revealed SLCO1B3's broad association with immune checkpoint genes (e.g., PD-L1, CTLA4), while OSCC-specific data demonstrated its inverse correlation with naïve B cell abundance—a potential mechanism for immune evasion.

Despite these findings, several limitations must be acknowledged. Our conclusions are derived predominantly from TCGA and GTEx datasets, necessitating validation in clinical cohorts with matched histopathological and survival data. Additionally, the molecular mechanisms underlying SLCO1B3-mediated suppression of naïve B cell infiltration remain unresolved. Future studies employing flow cytometry to characterize B cell subsets in SLCO1B3-high tumors, patient-derived organoids to model transporter-immune interactions, and in vivo genetic perturbation experiments will be critical to unravel these pathways.

Nevertheless, this study positions SLCO1B3 as a clinically actionable biomarker in OSCC, mechanistically linked to immunosuppressive TME remodeling through B cell dysregulation. These findings not only advance our understanding of OSCC immunobiology but also provide a rationale for developing biomarker-stratified immunotherapies targeting SLCO1B3-associated immune evasion mechanisms.

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