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Downregulated Expression of RIPOR3 Correlated with Immune Infiltrates Predicts Poor Prognosis in Oral Tongue Cancer

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Background: Tongue cancer is the most prevalent of head and neck squamous cell carcinomas, including base of tongue cancer (BOT) and oral squamous cell carcinoma of the mobile tongue (OTSCC). We aimed to investigate the role of RIPOR3 in tumorigenesis and its development as a potential prognostic biomarker for tongue cancer, especially OTSCC.


Material/Methods: Associations of expression, clinical pathologic features, and overall survival were analyzed by logistic regression, multivariate Cox analysis, and Kaplan-Meier methods. Gene set enrichment analysis (GSEA) and the CIBERSORT algorithm were performed to determine the correlation between RIPOR3 and tumor immune infiltration. cBioPortal was used for methylation and copy number variation (CNV) analysis. The Human Protein Atlas (HPA) and GSE31056 dataset were used for further external validation.

Results: RIPOR3 expression in OTSCC was significantly associated with various clinicopathological parameters. Kaplan-Meier survival analysis showed that OTSCC with low RIPOR3 expression had a worse prognosis than that with high RIPOR3 expression. Multivariate analysis revealed that lower RIPOR3 expression was an independent prognostic factor for poor prognosis. GSEA and Neighbor Gene Network analysis showed RIPOR3 expression was related with the modulation and function of the immune-related pathway. Methylation level and CNV analysis showed that the downregulated expression of RIPOR3 was significantly related to hypermethylation but not to CNV. Finally, high RIPOR3 expression was validated at the protein level using the HPA database and GSE31056 dataset.

Conclusions: These findings suggested that RIPOR3 might serve as a promising prognostic biomarker and is related to the immune cell infiltration of OTSCC.

Keywords: **Immunity • Prognosis • Squamous Cell Carcinoma of Head and Neck • Tongue Diseases**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/935055>

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Background

Head and neck tumors, 90% of which are composed of squamous cell carcinoma (HNSCC), are the sixth most common cancer worldwide [1]. Oral cancer accounts for 24% of HNSCC and is a major source of morbidity and mortality [2-4]. The tongue has a special anatomical position and is the main organ that participates in eating and taste feedback. When a patient encounters any discomfort, such as ulcers or pain, the patient should be evaluated for oral cancer at the first occurrence [5]; however, the incidence of advanced tumors (stage III-IV TNM) is still about 30% to 35% [6]. In patients under 40 years of age, the incidence of oral tongue cancer has increased significantly, by 60% [7]. Anatomically, the tongue is divided into the anterior two-thirds of the mobile body and the posterior third of the oropharyngeal area (the base of the tongue) by the V-shaped line, the sulcus terminalis. Pathologically, the cancer of the mobile tongue belongs to oral cancer, and tongue base cancer belongs to oropharyngeal cancer, which is completely different from oral cancer in terms of treatment methods, disease progression, malignancy degree, and clinical prognosis. In view of the high incidence of tongue cancer metastasis, avoiding cancer metastasis to oropharyngeal cancer, which has higher mortality, is important [2,8]. Therefore, it is of great significance to detect the occurrence of OTSCC as early as possible to prevent the tumor from spreading to the tongue base and distant metastasis and to improve the diagnosis rate and survival rate of patients.

RIPOR3, RIPOR family member 3, is also known as FAM65C, which presents in 2 subtypes produced by alternative splicing [9]. Previous studies indicated that RIPOR3 was involved in molecular alterations in HER2-positive and HER2-negative gastric cancers, including the somatic single nucleotide variant and somatic structural variation [10]. As a gain gene with high, frequent copy number variations (CNV), it is easier to detect in HER2+ samples, indicating that it promotes the progression of gastric cancer. Some studies have shown that as an age-related DNA methylation marker, RIPOR3 is in a gradual state of hypomethylation with aging. These findings suggest RIPOR3 may play an important role in various age-related diseases and cancer [11]. However, the relationship between RIPOR3 and clinical features of tongue cancer has not been studied.

Thus, we sought to comprehensively evaluate the influence of RIPOR3 expression on the prognosis of tongue cancer and explore the possible mechanism of this gene based on the data collected from The Cancer Genome Atlas (TCGA). Furthermore, we preliminarily explored the correlation of RIPOR3 with genetic and epigenetic mechanisms, including methylation level and CNVs. Finally, the Human Protein Atlas (HPA) database and GSE31056 dataset were used for further external validation.

Material and Methods

Analysis Data Acquisition and Bioinformatics Analysis

The gene expression profiles and clinical data (workflow type: HTSeq FPKM) were downloaded from the TCGA (<http://cancergenome.nih.gov/>) and TCGA Pan-Cancer (PANCAN) (<https://xena.ucsc.edu/>) datasets, including OTSCC data (140 cases) and OTSCC paired-sample data (normal samples and cancer samples from the same patient).

Survival Analysis and Cox Analysis

All statistical analyses were performed using R (v.3.6.3). The Wilcoxon rank-sum test and logistic regression were used to analyze the relationship between RIPOR3 and clinicopathological characteristics (age, sex, grade, stage, tumor size, regional lymph nodes, surgery treatment, and radiation therapy). Cox regression and the Kaplan-Meier method were used to analyze the relationship between overall survival (OS), progression-free interval (PFI), disease-specific survival (DSS), and the clinical features of patients with tongue cancer.

Gene Set Enrichment Analysis

Gene set enrichment analysis (GSEA) is a computational method that identifies whether a priori defined sets of genes show statistically concordant differences between 2 biological functions or phenotypes [12]. In our study, GSEA firstly generated an ordered list on the classification of all genes according to their correlation with RIPOR3 expression. GSEA was applied to calculate the statistically significant survival difference observed between RIPOR3 high- and low-expression groups. Gene sets with a false discovery rate (FDR) q -value < 0.05 were considered to be significantly enriched [13].

Immune Scores and Stromal Scores with RIPOR3 Expression

The Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) algorithm was used to calculate the immune and stromal scores [14]. ESTIMATE can predict tumor purity by calculating immune and stromal scores, thereby predicting the content of stromal cells and immune cells. Using the R packages “ggplot2”, “ggpubr”, and “ggExtra” and the Spearman method, we quantified the association of RIPOR3 expression with immune scores and stromal scores.

Correlation Analysis of RIPOR3 Expression with the Immune Response of 22 Tumor-Infiltrating Immune Cells

We analyzed the immune response of 22 tumor-infiltrating immune cells (TIICs) in OTSCC via the CIBERSORT algorithm

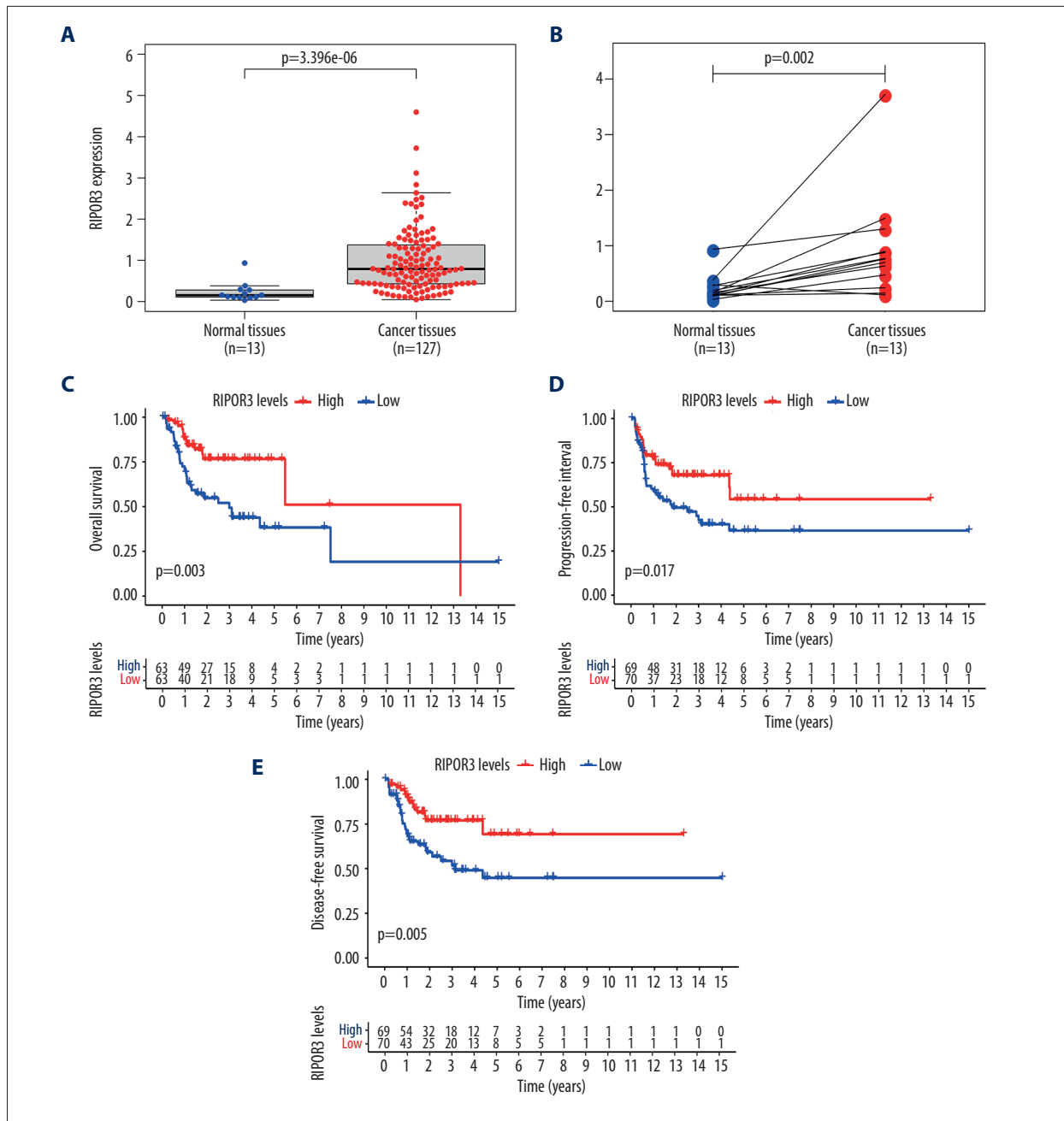


Figure 1. The differentiated expression of RIPOR3 and correlation with survival outcomes. **(A)** Differentiated expression of RIPOR3 in normal tongue tissue (n=13) and oral squamous cell carcinoma of the mobile tongue (OTSCC) (n=127) in the The Cancer Genome Atlas cohort ($P<0.0001$). **(B)** Paired heterogeneous analysis of RIPOR3 expression in normal and tumor samples derived from the same patient ($P=0.002$ by the Wilcoxon rank-sum test). **(C-E)** Kaplan-Meier survival analysis of the relationship between RIPOR3 mRNA expression and overall survival ($P=0.003$), progression-free interval ($P=0.017$) and disease-specific survival ($P=0.005$) in OTSCC cases.

Table 1. Univariate and multivariate Cox logistic regression analysis of RIPOR3 expression and other clinical pathological factors.

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age	1.007	0.977-1.038	0.625			
Sex	0.645	0.307-1.355	0.247			
Stage	1.470	0.974-2.219	0.067			
Grade	1.902	0.998-3.626	0.050	2.941	1.359-6.366	0.006
T	1.804	1.197-2.719	0.005	2.352	1.271-4.351	0.006
N	1.390	0.925-2.088	0.113	1.957	1.027-3.727	0.041
Surgery	0.212	0.080-0.566	0.002	0.113	0.030-0.431	0.001
Radiation	0.442	0.207-0.944	0.035	0.206	0.084-0.504	<0.001
RIPOR3	0.256	0.109-0.643	0.003	0.276	0.107-0.708	0.007

HR – hazard ratio; CI – confidence interval; T – tumor; N – lymph node.

(<http://cibersort.stanford.edu/>), with the algorithm running with its default signature matrix at 1000 permutations [15]. In addition to analyzing the general analysis of immune cell type, we analyzed the correlation between RIPOR3 expression and several immune cell markers to identify the potential subtypes of infiltrating immune cells. The Spearman method was used to determine the correlation coefficient.

cBioPortal

The cBioPortal (www.cbioportal.org/) is a comprehensive web resource that can visualize and analyze multidimensional cancer genomics data [16,17]. Based on the TCGA database, the CNVs, co-expression, and methylation data of the RIPOR3 gene were obtained from cBioPortal. When performing epigenetic analysis, we only performed statistical analysis of 127 tumor samples, and excluded 13 normal samples included in the 140 samples. The threshold of the z score of mRNA expression (RNA Seq V2 RSEM) was ± 2.0 .

Validation of the HPA and Gene Expression Omnibus Databases

The HPA database (<http://www.proteinatlas.org/>) provides information on the expression profiles of human proteins in various tissue types and organs. In the database, protein expression was scored by comprehensively evaluating the proportion of stained cells and staining intensity. Therefore, we compared the RIPOR3 protein level in normal and OTSCC tissues. The Gene Expression Omnibus (GEO) GSE31056 dataset was downloaded and analyzed to validate the accuracy and precision of the independent predictor from the TCGA cohort as a prognostic predictor for OTSCC.

Results

Tumorigenic Effect and Prognosis Value of RIPOR3 Low Expression

We obtained the data of TCGA, processed the expression data of mRNA, and found that the transcripts of RIPOR3 in OTSCC tumor tissue were significantly higher than that in normal tissue ($P=3.396E-06$) (Figure 1A). The trend of gene overexpression was also observed in OTSCC paired samples ($P=0.002$) (Figure 1B) by the Wilcoxon rank-sum test.

Survival Outcomes and Prognostic Value of RIPOR3

All tongue cancer patients were divided into 2 groups based on the median expression value of RIPOR3: a high-expression group and a low-expression group. Kaplan-Meier survival analysis showed the high-expression group in OTSCC had a significantly positive correlation with OS, PFI, and DSS (Figure 1C-1E). The univariate analysis revealed that low RIPOR3 expression was significantly correlated with poor OS (hazard ratio [HR] 0.256, 95% confidence interval [CI] 0.109-0.643, $P=0.003$; Table 1). By multivariate analysis, RIPOR3 remained independently associated with OS, with an HR of 0.276 ($P=0.007$), along with advanced grade and tumor size, regional lymph node, surgery treatment, and radiation therapy (Figure 2D).

Clinical Characteristics and Multivariate Analysis with RIPOR3 Expression

Subsequently, we examined the correlation between gene and clinicopathological features, and the results showed that the expression of RIPOR3 was significantly correlated with tumor stage ($P=0.029$), tumor size ($P=0.005$), and tumor histological grade ($P=0.022$) (Figure 2A-2C). As shown in Table 1,

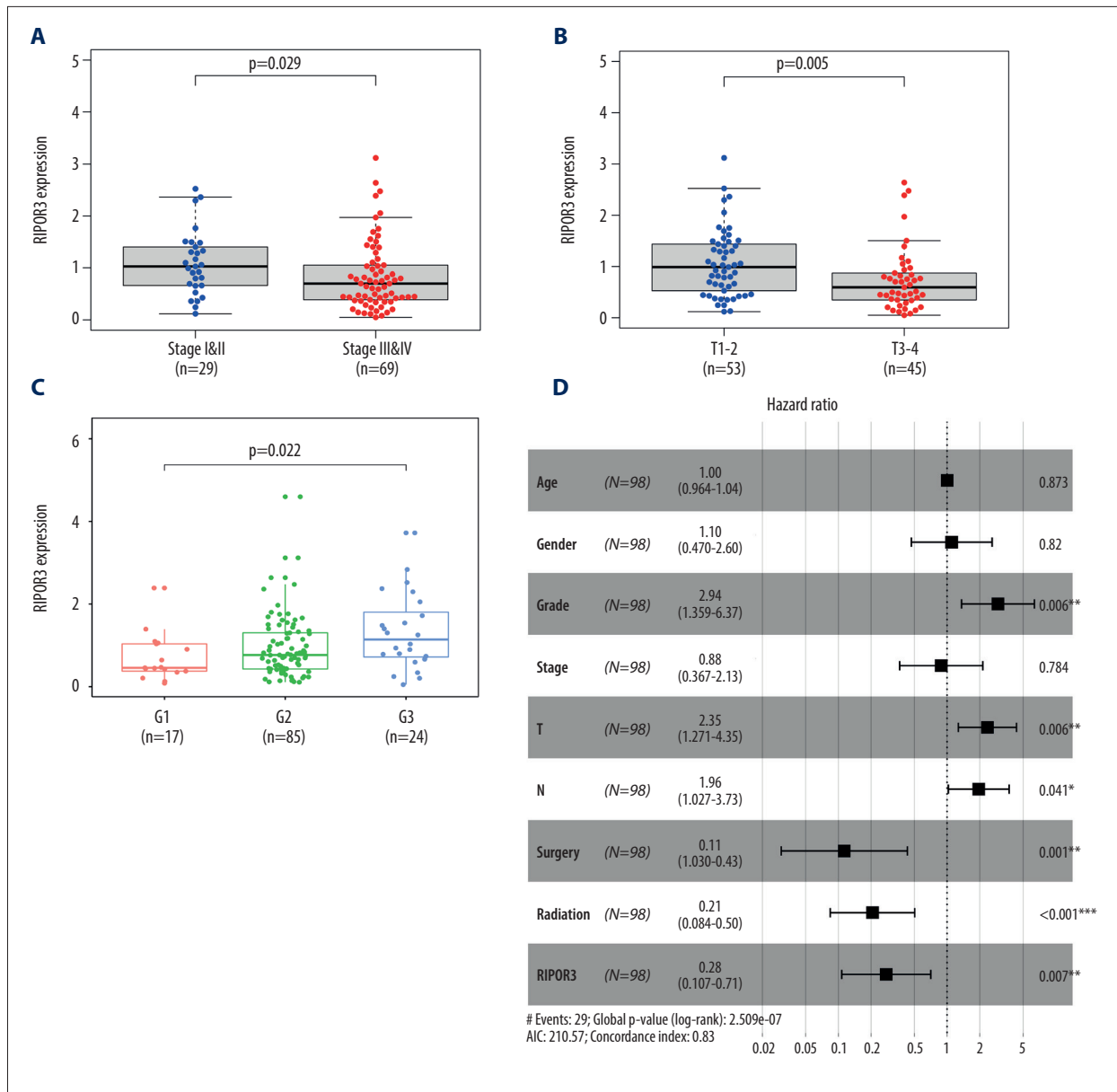


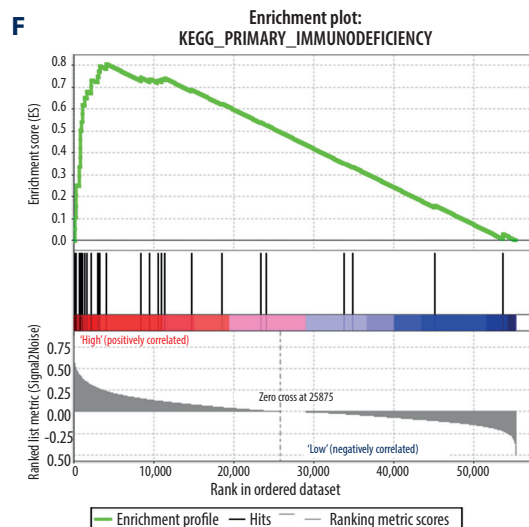
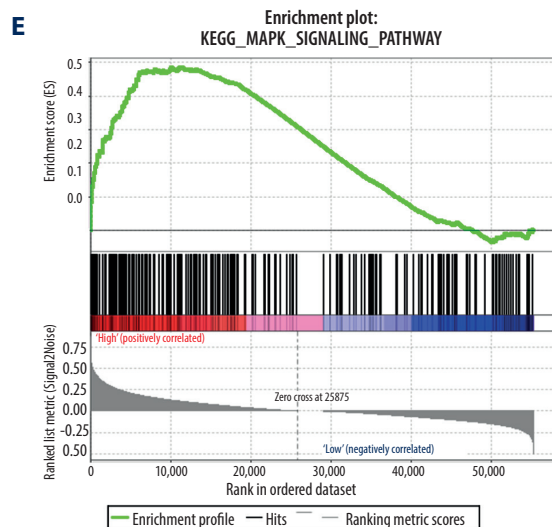
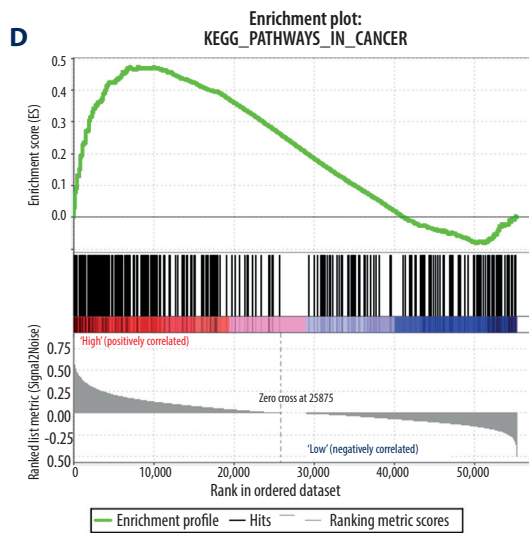
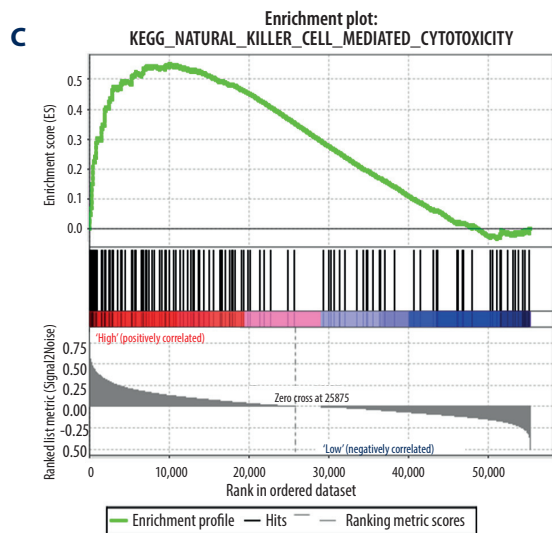
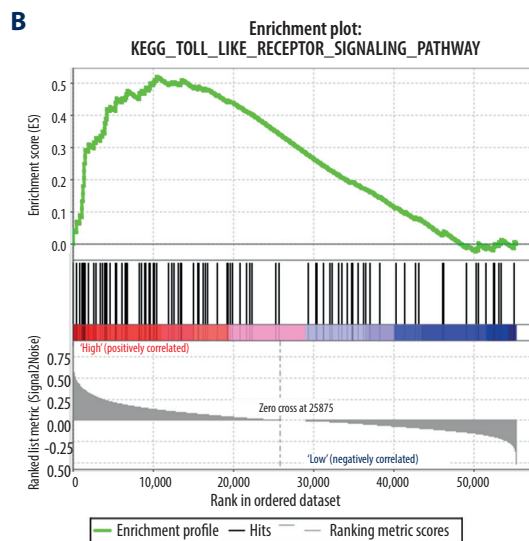
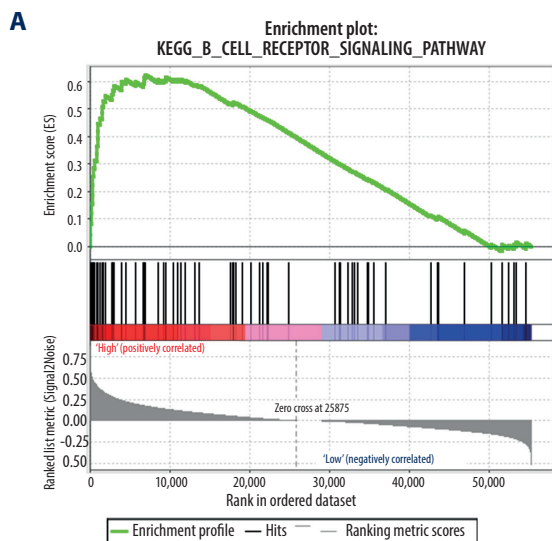
Figure 2. Correlations between RIPOR3 mRNA levels with oral squamous cell carcinoma of the mobile tongue (OTSCC) clinical characteristic in the The Cancer Genome Atlas cohort. **(A)** Stage (stage I&II, n=29; stage III&IV, n=69). **(B)** Tumor size (T1-2, n=53; T3-4, n=45). **(C)** Grade (G1, n=17; G2, n=85; G3, n=24). **(D)** Multivariate Cox analysis of RIPOR3 expression and clinical characteristics in OTSCC (n=98). (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

univariate Cox regression analysis showed that grade, tumor size, regional lymph nodes, surgery treatment, and radiation therapy were significantly correlated with the rate of OS. In multivariate analysis, RIPOR3 expression was an independent prognostic factor (**Figure 2D**).

GSEA Analysis of RIPOR3-Related Signaling Pathway

To identify signaling pathways that were differentially activated in OTSCC, we conducted GSEA between RIPOR3 datasets

with low and high expression. We selected the most significantly enriched signaling pathways based on their FDR q-val in the high-expression samples, but no significant pathway was found in the low-expression group. As shown in **Figure 3A-3I**, the B-cell receptor signaling pathway, T-cell receptor signaling pathway, natural killer cell-mediated cytotoxicity, pathway in cancer, primary immunodeficiency, TGF- β signaling pathway, Toll-like receptor pathway, JAK-STAT signaling pathway, and MAPK signaling pathway were differentially enriched in the RIPOR3 high-expression phenotype.



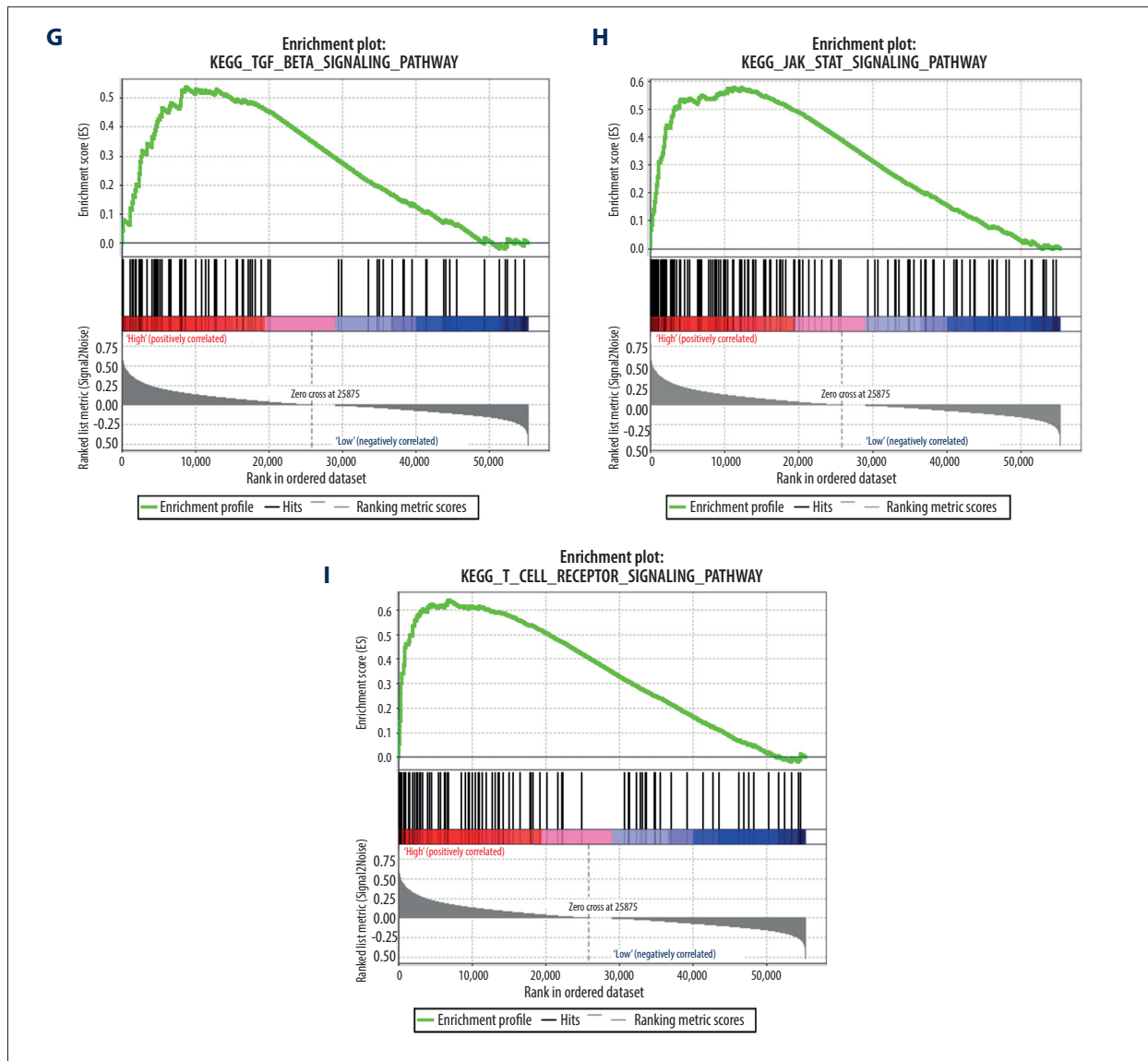


Figure 3. (A-I) Gene Set Enrichment Analysis. Each line represents 1 particular gene set and high-expression genes are in the left, approaching the origin of the coordinates; by contrast, the low-regulated are on the right of the X-axis. Only gene sets with $NOM P < 0.05$ and false discovery rate (FDR) $q < 0.05$ were considered significant. Genes associated with a high-risk phenotype were significantly enriched with the immune-related pathway.

Association with the Expression of RIPOR3 and Tumor Microenvironment

From the results of GSEA, it was seen that the RIPOR3 gene was significantly enriched in the immune-related signaling pathway; therefore, we divided the tumor samples into high- and low-expression groups based on the median value of RIPOR3 expression and further evaluated the relationship between the RIPOR3 and tumor microenvironment by quantifying the tumor stromal cell score, immune cell score, and RIPOR3 expression level. More interestingly, as shown in **Figure 4C and 4D**, the scores of stromal cells and immune cells were positively

correlated with the expression of RIPOR3 in the tumor groups ($R = 0.62$, $P < 2.2 \times 10^{-16}$; $R = 0.47$, $P = 4.7 \times 10^{-8}$), which indirectly indicated that the gene was related to the tumor microenvironment and immune activity.

TIICs and RIPOR3 Expression

To further confirm the correlation of RIPOR3 expression with the immune microenvironment, we analyzed the content difference of 22 TIICs in the 2 groups with the CIBERSORT algorithm. The results showed that the fraction of naive B cells and resting mast cells increased significantly in the high-expression

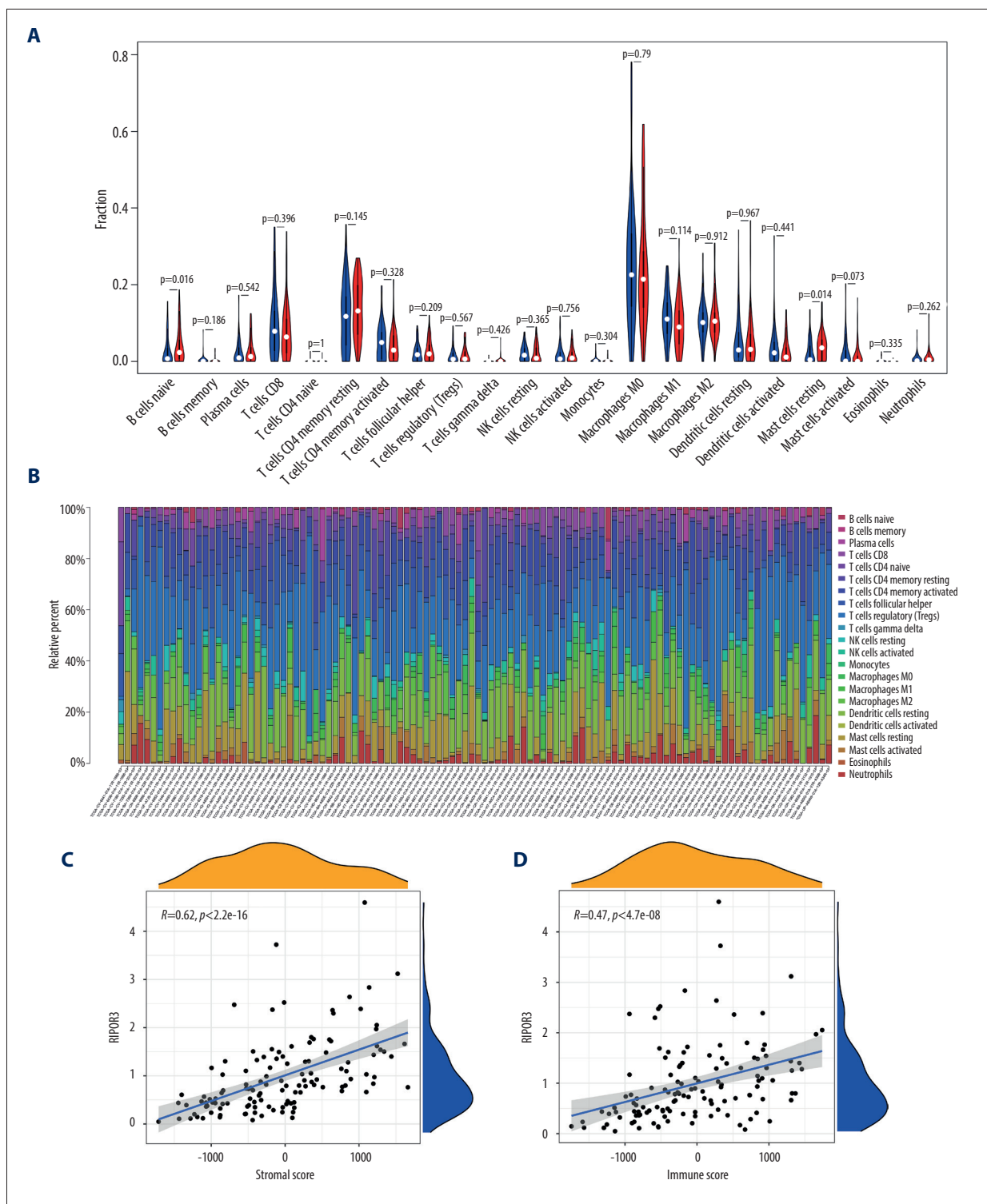
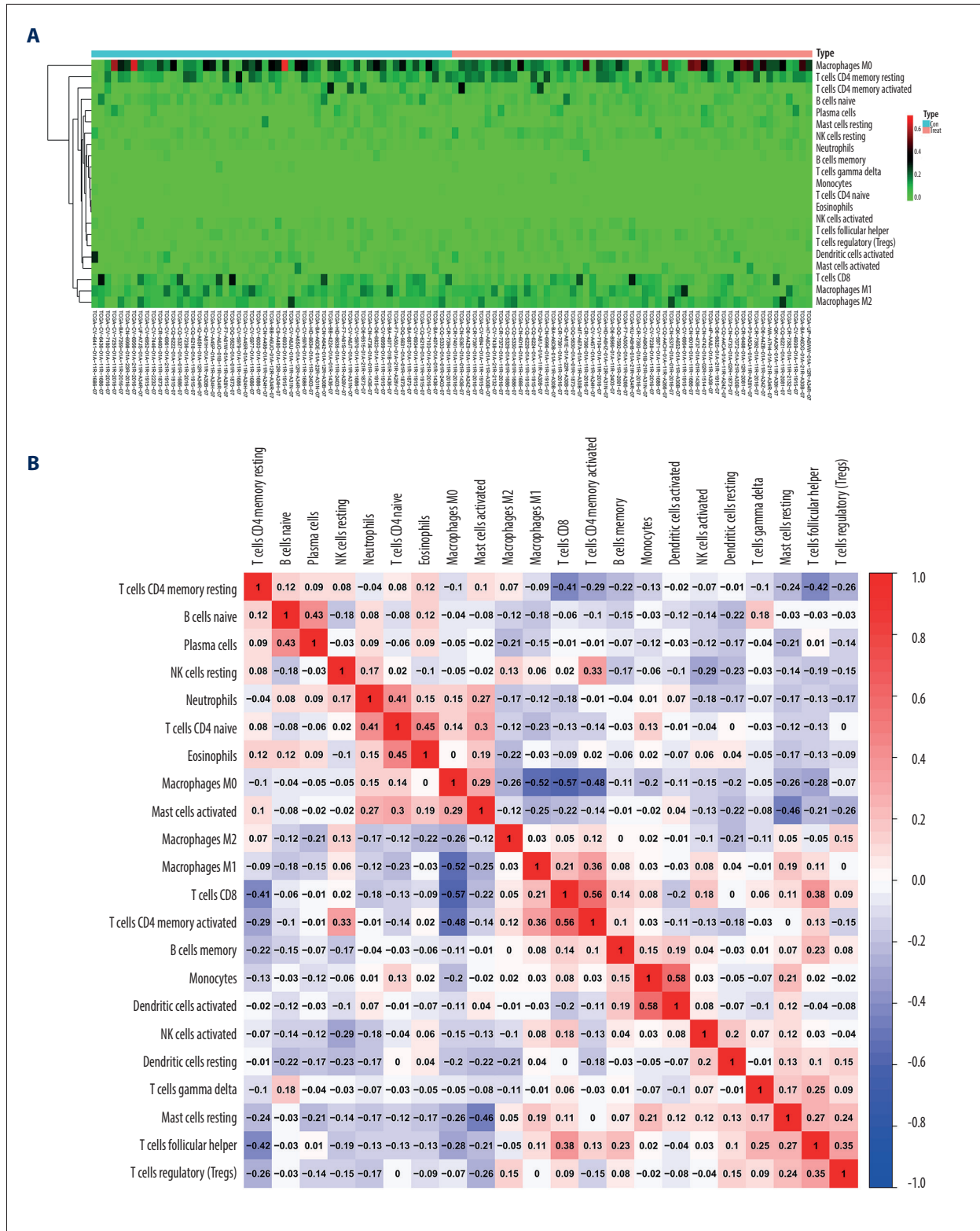


Figure 4. Correlation of RIPOR3 with tumor-infiltrating immune cells (TIICs). **(A)** Violin plot showing the ratio differentiation of 22 kinds of immune cells between oral squamous cell carcinoma of the mobile tongue (OTSCC) samples ($n=110$), which was filtered by the CIBERSORT algorithm with $P<0.05$, with low or high RIPOR3 expression relative to the median of the RIPOR3 expression level. **(B)** Barplot showing the proportion of 22 kinds of TIICs in OTSCC tumor samples. Column names of the plot were sample ID. **(C, D)** Scatter plot showing the correlation of the stromal cell score and immune cell score with RIPOR3 expression ($P<0.05$). (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).



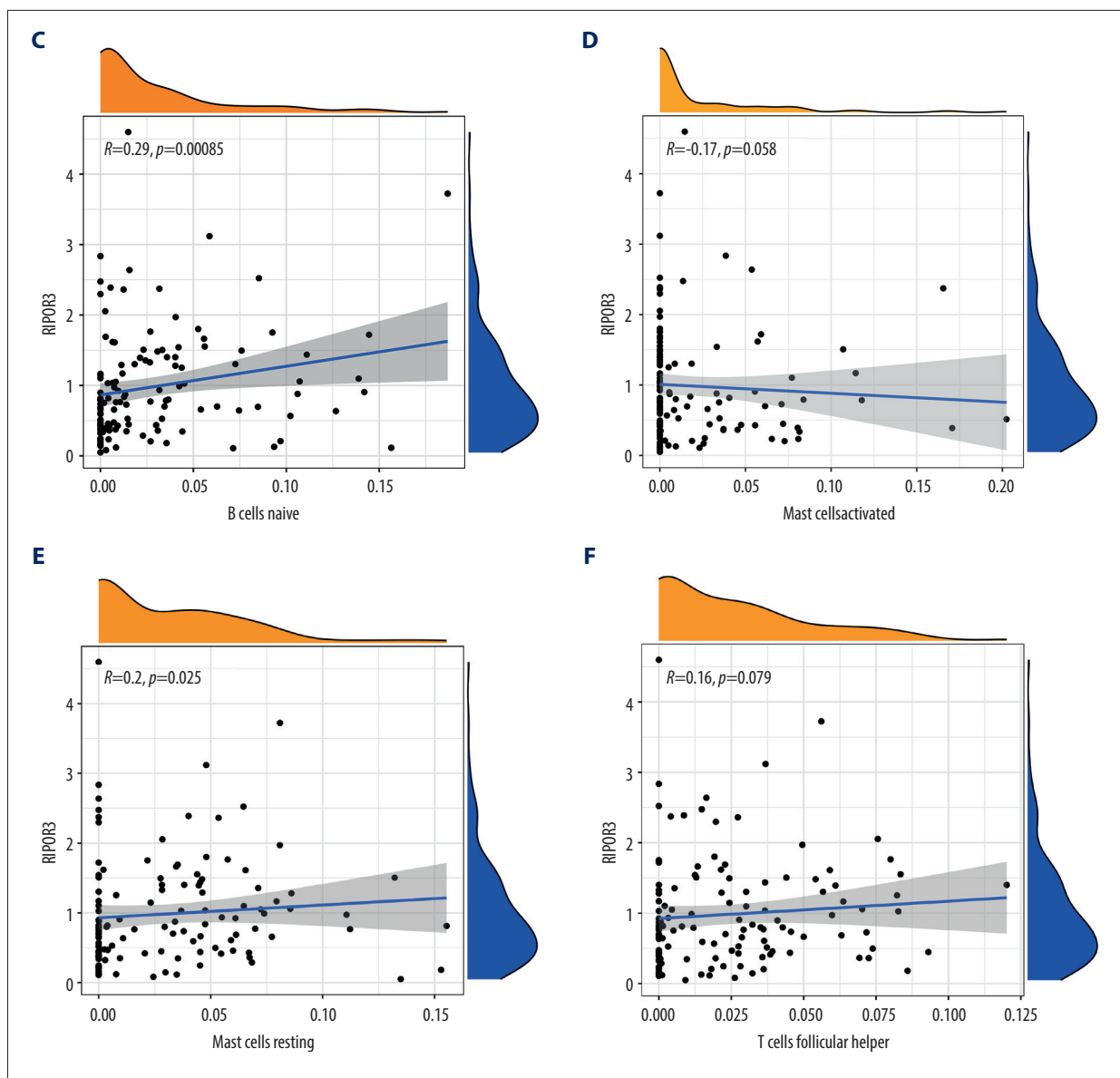


Figure 5. Immune cell infiltration patterns and correlation of high and low RIPOR3 expression groups with differential immune cells. (A) Heatmap of tumor-infiltrating immune cells (TIICs) in low-expression ($n=55$) and high-expression groups ($n=55$). (B) Heatmap showing the correlation between 22 TIICs and numeric value in each tiny box indicating the P value of correlation between the 2 kinds of cells. The shade of each tiny color box represents corresponding correlation value between 2 cells, and Pearson coefficient was used for significance test. (C-F) Scatter plot showing the correlation of TIIC proportion with RIPOR3 expression. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

group (Figure 4A), which was similar and complementary to the GSEA function analysis results. Figure 4B shows the barplot of the proportion of the 22 TIICs in each sample of the high-expression group and low-expression group. The correlation heatmap of immune cells in patients with OTSCC, as shown in Figure 5A and 5B, provided us with the interaction between the expression of different immune cells. It can be seen that there were significant positive and negative correlations between a variety of immune cells, such as CD8 T cells with activated

memory T cells, activated dendritic cells with monocytes, and CD8 T cells with macrophages M0 (Figure 5B). In addition, we determined that naive B cells ($R=0.29$, $P=0.00085$) and resting mast cells ($R=0.2$, $P=0.025$) were significantly positively correlated with RIPOR3 expression and activated mast cells ($R=-0.17$, $P=0.058$) and follicular helper T cells ($R=0.16$, $P=0.079$) showed certain correlations (Figure 5C-5F). These results further supported that the levels of RIPOR3 affected the immune-dominant status for the tumor microenvironment.

Relationship Between Immune Cells and Clinical Features

Based on the above confirmed correlation between RIPOR3 expression and tumor microenvironment and immune cell infiltration, we further confirmed, by analyzing the relationship between immune cells and clinicopathological characteristics, that immune cells infected by RIPOR3 did participate in and played a certain role in the occurrence and development of OTSCC. Resting CD4 memory T cells and lymph node status ($P=0.0019$), regulatory T cells (Tregs) and tumor size ($P=0.025$), and resting dendritic cells and tumor size ($P=0.0017$) were significantly correlated (Figure 6A-6C).

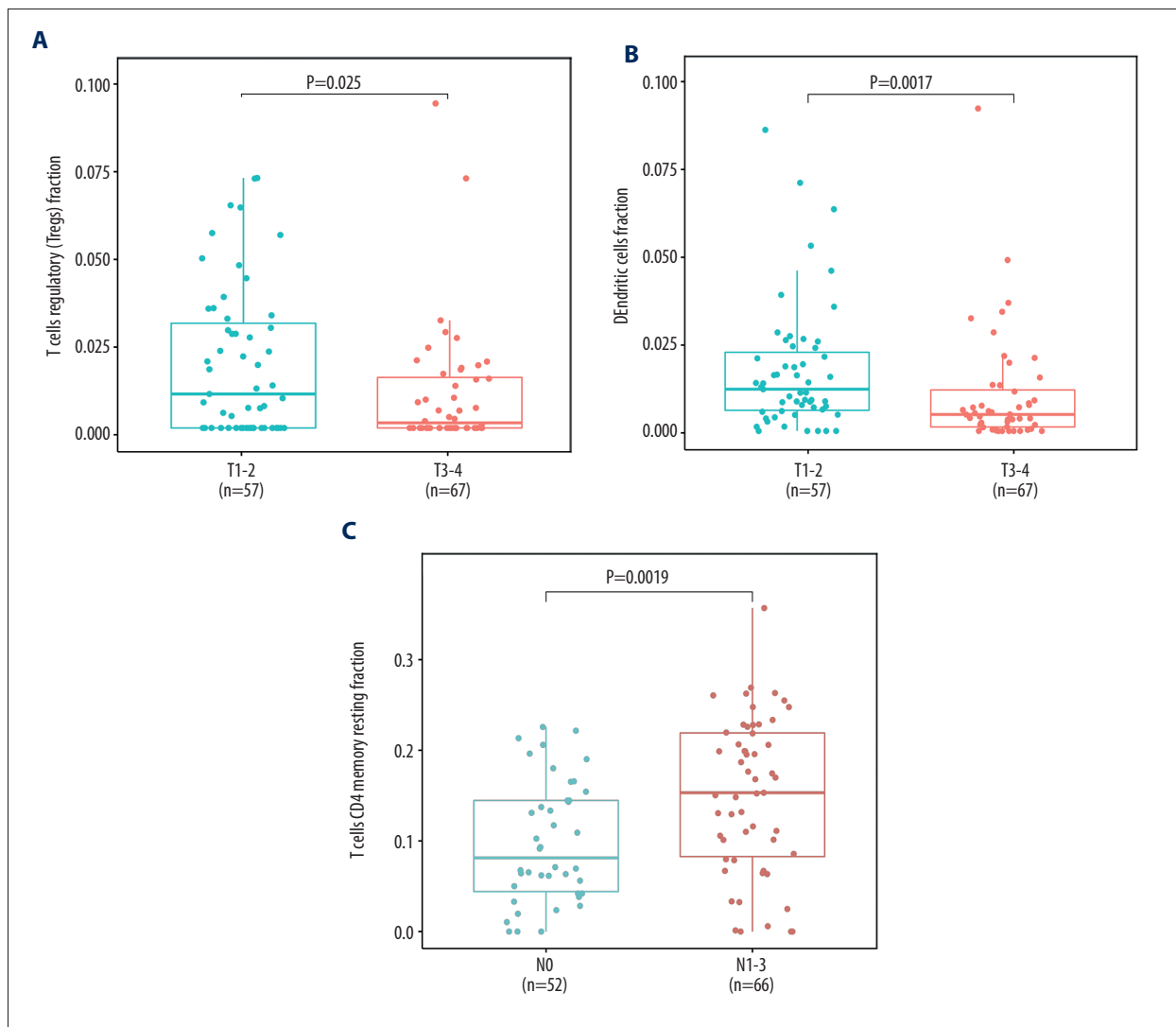
Neighbor Genes, CNVs, Methylation, and Interaction Analyses of RIPOR3 in Patients with OTSCC

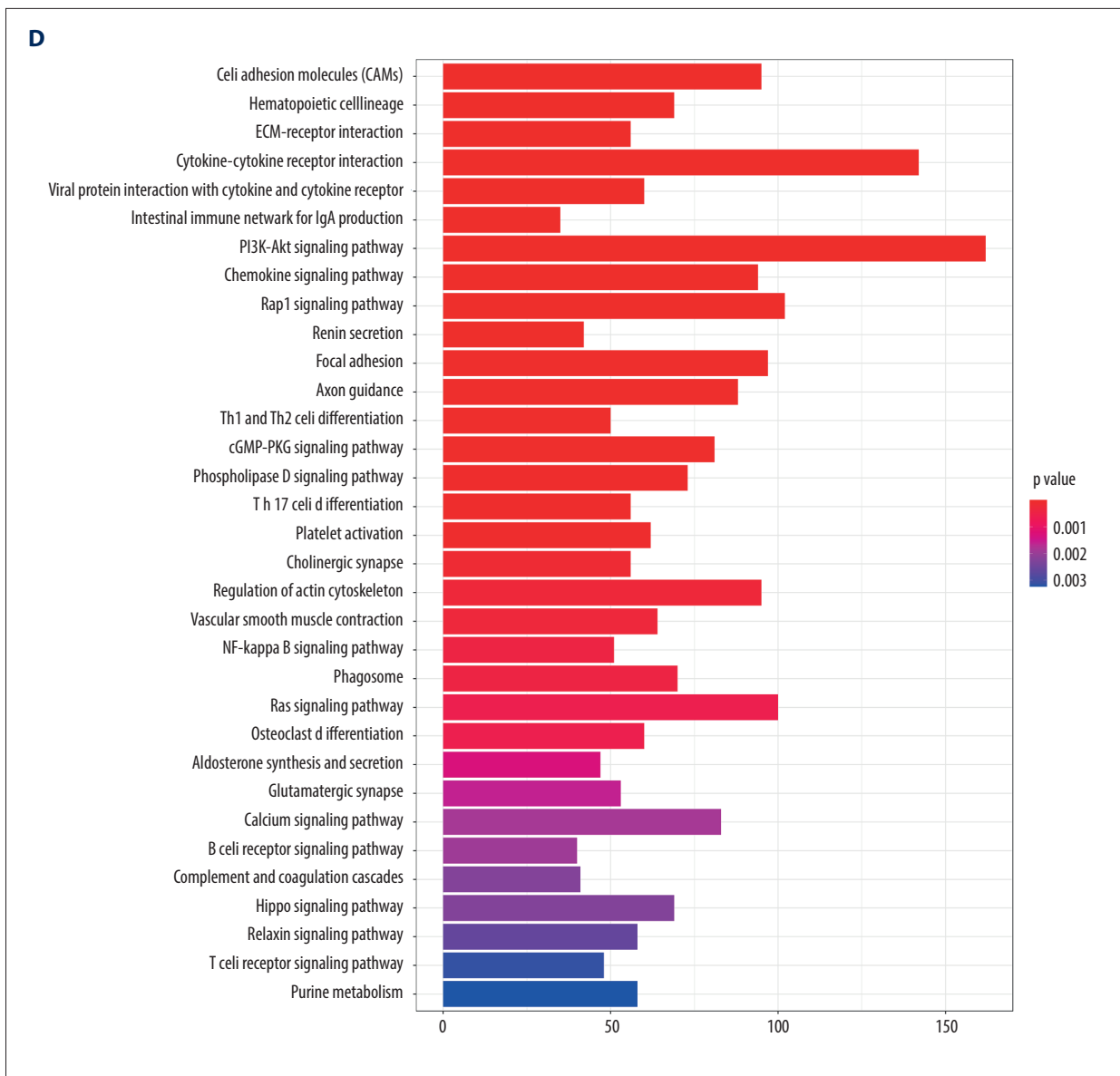
The most frequently altered neighbor genes (FDR q -value <0.05) associated with RIPOR3 expression were isolated

with cBioPortal. Enrichment analysis of these related genes showed that multiple functional enrichment was significantly enriched in immune-related pathways, such as Th1 and Th2 cell differentiation, Th17 cell differentiation, B-cell receptor signaling pathway, and T-cell receptor signaling pathway. The methylation and CNV analysis data of 127 tumor samples of OTSCC were also obtained from cBioPortal. As shown in Figure 7, Spearman and Pearson analyses both showed that the mRNA level of RIPOR3 was significantly negatively correlated with the degree of methylation, and RIPOR3 expression in the hypermethylation group was lower (Figure 7B). However, no correlation between the change of copy number type and gene expression was found (Figure 7A).

Validation of the HPA and GSE31056 Databases

We evaluated the expression of RIPOR3 protein in 20 cancer tissues from the HPA database and found that most cancer





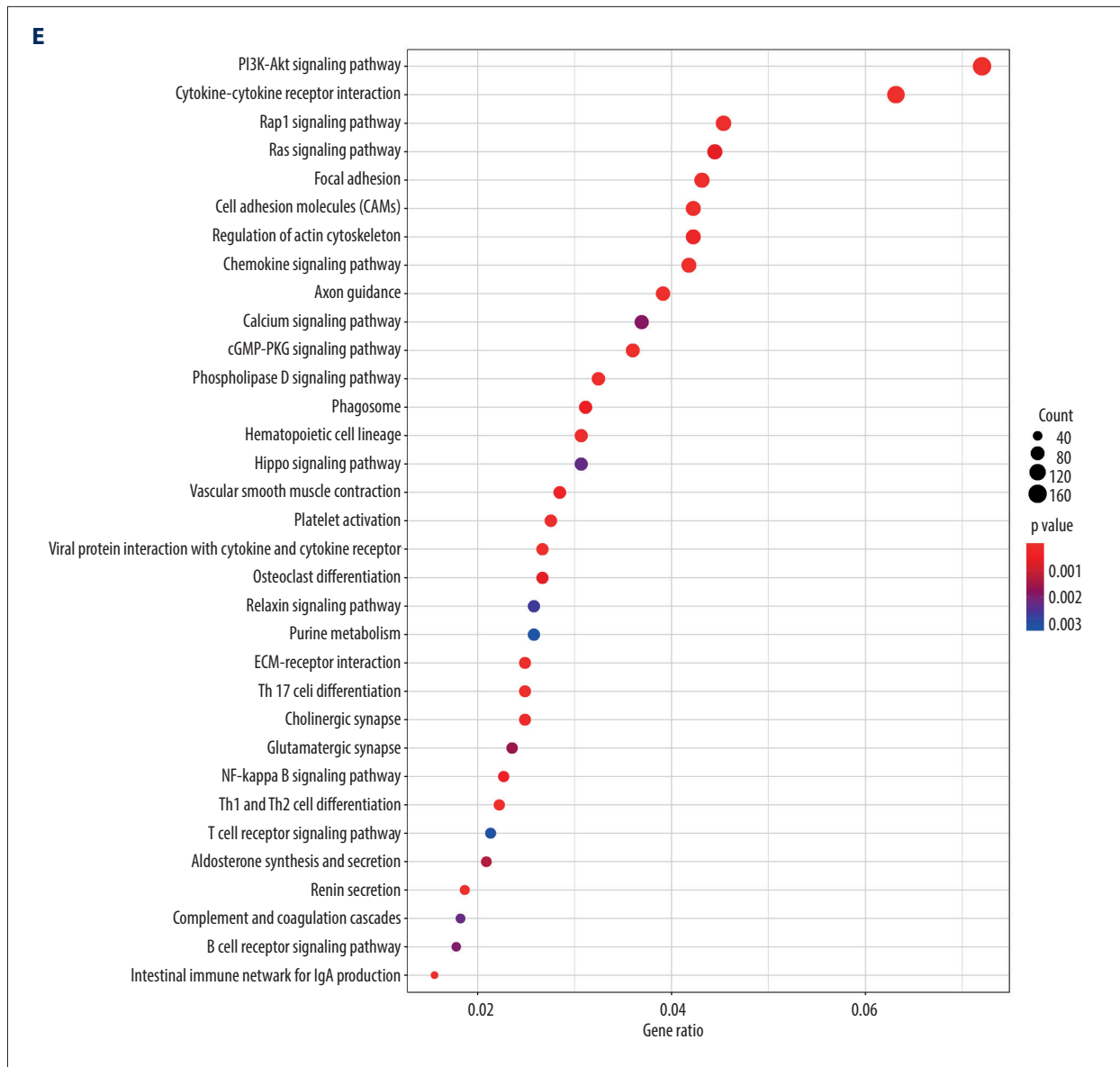


Figure 6. Correlations between immune cells level and clinicopathological parameters of oral squamous cell carcinoma of the mobile tongue (OTSCC) patients. (A, B) Differential expression of T regs and dendritic cells resting fraction were significantly negatively related to tumor size ($P=0.025$, $P=0.002$; T1-2, $n=57$, T3-4, $n=67$), while (C) CD4 memory resting T cells were significantly positively correlated with lymph node size ($P=0.002$, $N_0=52$, $N_{1-3}=66$). (D) The barplot of KEGG enrichment analysis of most frequently altered neighbor genes in OTSCC with RIPOR3. (E) The dotplot of KEGG enrichment analysis of most frequently altered neighbor genes in OTSCC with RIPOR3. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

tissues displayed weak to moderate nuclear and occasional cytoplasmic positivity, and a relatively higher protein expression ratio in head and neck cancer and thyroid cancer (Figure 8A). As shown in Figure 8C and 8D, negative and moderate RIPOR3 staining were observed in the normal tongue tissue, while low (stage II, T2) or moderate (stage III, T3) protein expression was reflected in tumor tissues (Figure 8E, 8F). We used the GSE31056 dataset as an external cohort to identify the differential expression level of RIPOR3 in tumor and normal tissues

(Figure 8B). The results were consistent with the conclusions of the TCGA cohort analysis, showing significantly higher expression in tumor than in normal tissues ($P=0.005$).

Discussion

RIPOR3, RIPOR family member 3, is located on chromosome 20 [18], with broad expression in the bone marrow, kidney, and

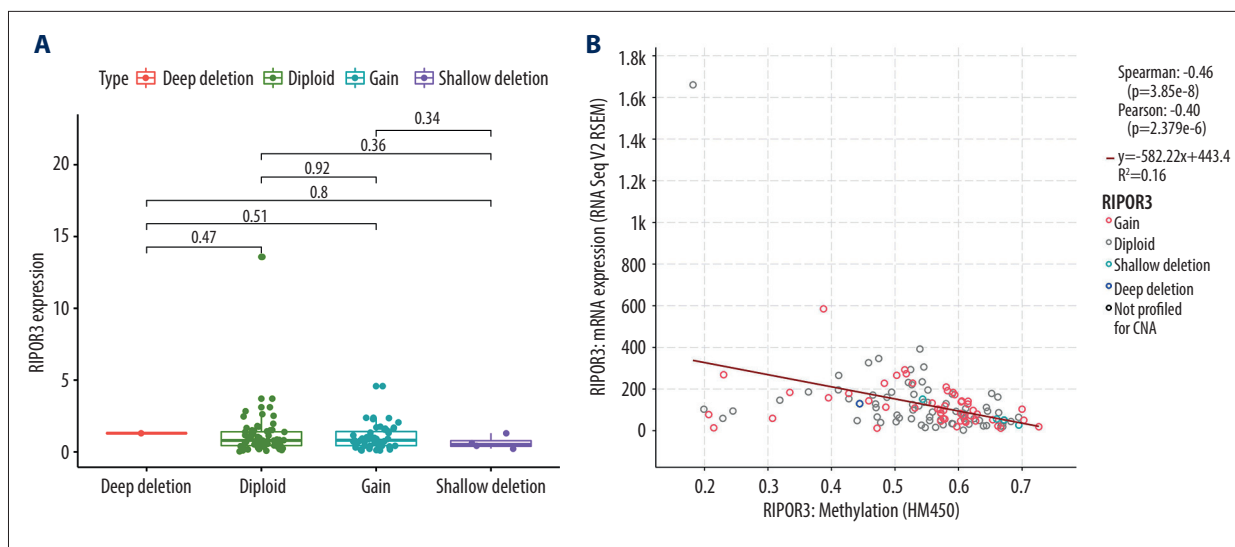


Figure 7. Correlation of RIPOR3 expression levels with genetic and epigenetic mechanisms. **(A)** Copy number variation. **(B)** Methylation level. The red line in the plot was fitted with a linear model indicating the proportion of tropism of the methylation level along with RIPOR3 expression, and the Spearman coefficient was used for the correlation test. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

22 other tissues. Protein FAM65C, a member of the extracellular region, regulates cellular processes in response to stimuli [19]. At present, the reports in the literature on the RIPOR3 gene are limited. Recently, Zhou analyzed the lncRNA-miRNA-mRNA ceRNA network in squamous cell carcinoma of the tongue and found some mRNA, lncRNA, and miRNA are significantly related to survival, including RIPOR3; this was one of the few reports on the relationship between this gene and tongue cancer [20]. In view of the high incidence of tongue cancer, differential tumor prognosis in the anterior tongue (mobile body of the tongue) and base of the tongue, and the effect on the quality of life of patients, we systematically conducted the first-ever analysis of the role of RIPOR3 in tongue cancer, especially OTSCC, in the present study.

We found that the RIPOR3 expression in samples from patients with OTSCC was significantly higher than that in normal samples and also showed statistically significant differences in matched samples, as verified in the GEO and HPA databases. It was identified that an increased RIPOR3 expression contributed to favorable OS; the PFS also showed a higher proportion of survival in the high-expression group with the same time from randomization to tumor progression. Similarly, DSS reflected that patients who died of OTSCC had a shorter survival time in the low-expression group. Multivariate analysis also showed that high RIPOR3 expression was an independent predictive factor associated with favorable OS in cohorts. In addition, low expression of RIPOR3 in OTSCC was positively related with advanced tumor stage and regional lymph nodes but negatively correlated with tumor grade, which could be seen from hazard ratio confidence intervals, indicating that different clinical traits had

multiple adaptive reactions to RIPOR3. GSEA analysis showed that B-cell and T-cell receptor signaling pathways, natural killer cell-mediated cytotoxicity, pathway in cancer, and primary immunodeficiency in KEGG were enriched in the high RIPOR3 expression phenotype pathway, which suggested that this gene might be related to the immune-related regulatory pathway. Since the research on the function of the RIPOR3 gene was quite limited, we evaluated 2 other members of the RIPOR3 family, FAM65A and FAM65B. Megrelis et al found that the expression and phosphorylation of FAM65B can well regulate the number of active RhoA, which was conducive to the best movement of T lymphocytes and enhanced immune function [21]. Froehlich et al proved that FAM65B was related to T-cell migration and proliferation [22]. FAM65A played a role in regulating Golgi reorientation during cell migration [23]. Relevant literature showed that immune cells were an important part of tumor tissue, and the infiltration of immune cells varied greatly among different tumor types and histological subtypes [24]. Therefore, based on the immune pathway enriched by GSEA and the role of FAM65 family members in cell migration and immunity, we speculated that the relatively longer survival of tumor patients with high expression of RIPOR3 was due to the fact that immune cells play a certain role, thus affecting the progression of the disease.

Another study showed strongly associated gene expression in different immune cells [25]. We analyzed the relationship between the RIPOR3 gene in the tumor microenvironment and immune cell infiltration and found that diverse immune marker sets and immune infiltration levels in OTSCC were correlated with high RIPOR3 expression and the contents of naive B cells and resting mast cells were significantly increased

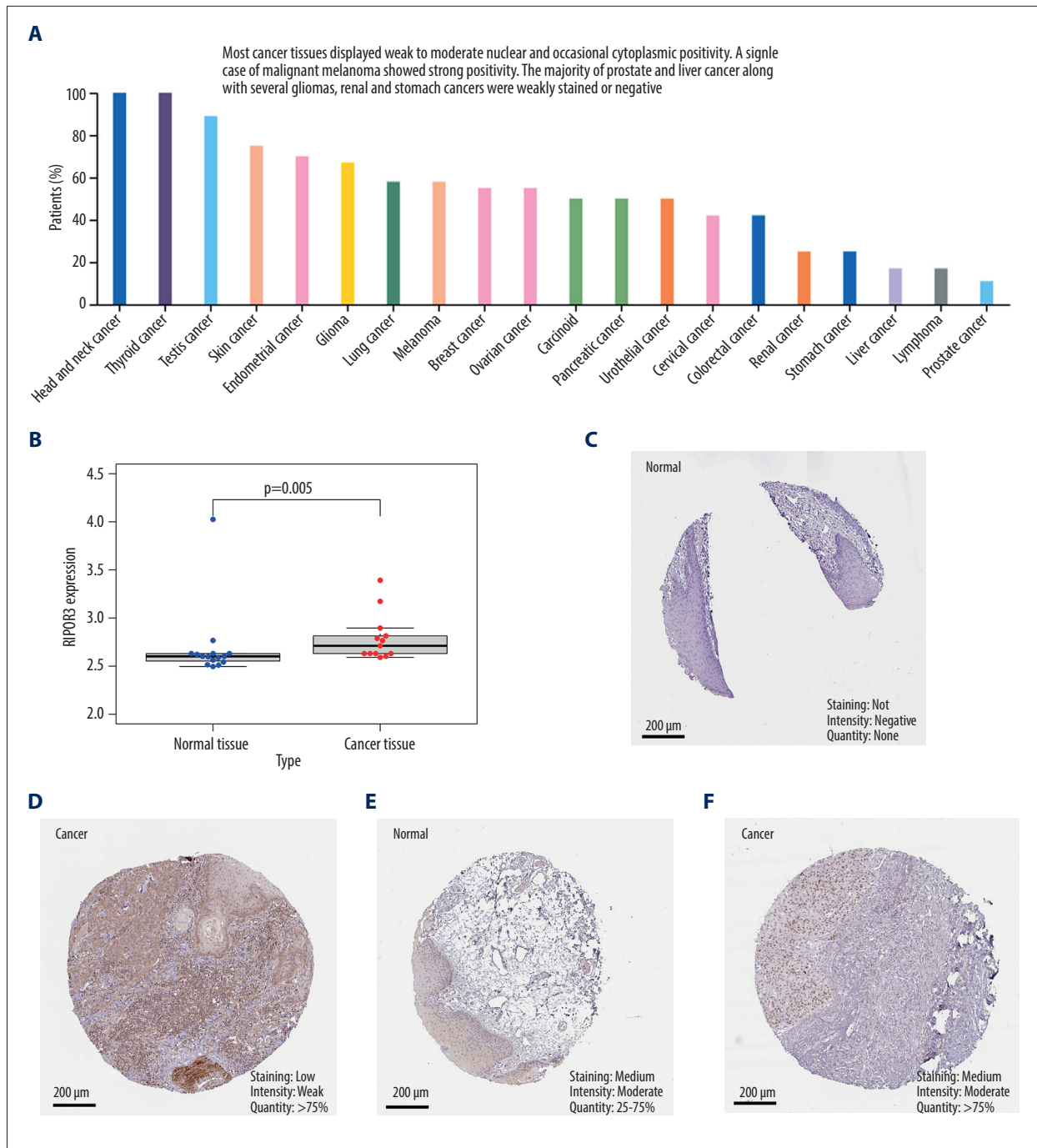


Figure 8. Immunohistochemical analysis of RIPOR3 protein expression in 20 major malignant tumors and expression levels in normal and oral squamous cell carcinoma of the mobile tongue (OTSCC) patients. **(A)** Changes of protein expression in different malignant tumors. **(B)** Difference in RIPOR3 expression between normal tongue tissues and OTSCC patients from GSE31056 dataset. **(C)** Negative RIPOR3 expression in normal tongue tissue. **(D)** Low protein expression in cancer tissues (stage II, T2). **(E)** Moderate RIPOR3 expression in normal tongue tissue. **(F)** Moderate RIPOR3 expression in OTSCC tissue (stage III, T3).

in patients with upregulated expression. In addition, the correlation heatmap showed some strong correlations between immune cells, and we found that certain immune cells were significantly associated with clinical traits; for instance, different T-cell subtypes were remarkably correlated with tumor size, regional lymph nodes, and tumor stage. A strong correlation between the expression of immune signals in different types of immune cells showed diverse but quite predictable and consistent tumor immune infiltration [26]. Finally, function enrichment analysis of differentially expressed neighbor genes related to RIPOR3 showed that multiple immune pathways played a certain role in tumor progression.

Genetic and epigenetic mechanisms play an important role in the process of tumorigenesis [27]. Genetic mechanisms mainly include aberrant gene expression. Epigenetic mechanisms include methylation modification and post-transcriptional regulation [28]. Gene methylation will block the initiation of transcription and prevent the transcription process, which is characterized by hypomethylation that activates gene transcription [29], while hypermethylation hinders gene transcription and leads to gene silencing [30]. Therefore, we preliminarily studied the possible mechanism of RIPOR3 expression from the CNV and methylation level of the gene. Different types of CNVs had no significant effect on gene expression, but DNA methylation was significantly negatively correlated with the expression of RIPOR3. The hypermethylation level inhibited the transcription of mRNA and reduced RIPOR3 expression. We speculated that the downregulated expression of RIPOR3 in OTSCC patients with poor prognosis was partly due to hypermethylation modification. Through the HPA and

GEO databases, it was confirmed that the gene had higher expression in tumor tissues and increased RIPOR3 contributed to favorable OS.

Therefore, we suggested that RIPOR3 may have a potential effect on tumor immunology. When the carcinogenic factors appear, the gene will be differentially expressed in patients, affecting and regulating immune-related pathways, thus playing an anti-tumor role. Therefore, based on the above results, the RIPOR3 gene may be a favorable gene that affects and regulates varieties of immune cells and is a promising tumor biomarker.

Our research had some limitations, such as a small sample size, incomplete clinical data, and lack of some etiology. Therefore, the molecular mechanisms involved may be the focus of our future work.

Conclusions

Taken together, our study results suggested that RIPOR3 might serve as a promising prognostic biomarker and is correlated with immune cell infiltration and DNA methylation. These results may provide new insights for the prognosis and immunotherapy of tongue cancer.

Declaration of Figures' Authenticity

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