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RESEARCH ARTICLE

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Identification of autophagy-related biomarker and analysis of immune infiltrates in oral carcinoma

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Abstract

Background: Autophagy plays a vital role in the progression of the tumor. We aimed to investigate the expression, prognostic value, and immune infiltration of autophagy-related genes in oral carcinoma via bioinformatics analysis.

Methods: The microarray datasets (GSE146483 and GSE23558) of oral carcinoma were downloaded from Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) between normal and diseased groups were identified by the Limma package. The screened autophagy-related gene was further validated by the human protein atlas (HPA) database, TCGA database, and GSE78060 dataset.

Results: A total of 18 upregulated (top 10: EGFR, TNF, FADD, AURKA, E2F1, CHEK1, BRCA1, BIRC5, EIF2AK2, and CSF2) and 31 downregulated (top 10: MAP1LC3A, PARK2, AGT, IGF1, TP53INP1, CXCL12, IKBKB, SESN1, ULK2, and RRAGD) autophagy-related (DEGs) were identified, and FADD was found to be related to the prognosis of oral cancer patients. Gene set enrichment analysis indicated that FADD-associated genes were significantly enriched in immune-related pathways. Moreover, correlation analysis revealed that FADD expression was associated with immune infiltrates. Upregulation of FADD is associated with poor survival and immune infiltrates in oral cancer.

Conclusion: We speculated that FADD is involved in the immune regulation of oral cancer, as well as autophagy.

KEYWORDS
autophagy, bioinformatics, FADD, immune infiltrates, oral cancer

1 | INTRODUCTION

Oral carcinoma is the most prevalent head and neck squamous cell carcinoma in the world, with about 10,800 new deaths and around 53,000 new cases in 2019.[1] It is also the most common histological subtype of all malignancies of the oral cavity.[2] Previous reports revealed that human papillomavirus infection, tobacco consumption, alcohol consumption, and betel nut chewing are the primary etiological factors involved in the development of oral carcinoma.[3-5] Although the considerable achievements in the treatment and diagnosis of oral carcinoma, the five-year survival rate of oral carcinoma sufferers is still less than 60% and the mortality of oral carcinoma

has not significantly improved.[6] Thus, it is important to identify potential molecular biomarkers and therapeutic targets for early diagnosis, prevention, and treatment of oral carcinoma to improve clinical therapeutics.

Autophagy is a catabolic biological phenomenon that maintains cell homeostasis through the degradation of damaged cellular components that fuse with lysosomes to form autolysosomes and is a primary cellular process that is involved in cancer, development, cellular component clearance, aging, and cell stress.[7,8] Autophagy dysregulation is involved in various human diseases, including tumors.[8] Recently, many reports have demonstrated that abnormal autophagy plays an important role in various malignant tumors, such as non-small-cell lung carcinoma, renal cell cancer, liver cancer, and colorectal cancer.[9-12] It has been demonstrated that autophagy plays an important role in the development of oral cavity carcinoma. For example, NKX2-3, FADD, PARK2, and EGFR were identified as potential prognostic autophagy-related markers during head and neck squamous cell carcinoma tumorigenesis.[13,14] SPHK1 ATG12, BID, and NKX2-3 were differentially expressed between normal tissues and oral cancer tissues and identified as potential biomarkers for oral squamous cell carcinoma prognosis.[15,16] These reports implied that autophagy-related genes might be used as a reliable biomarker for sufferers with oral carcinoma. The autophagy-related genes might also serve as therapeutic targets for oral cavity carcinoma therapy.

This research aimed to identify autophagy-related genes and potential pathways in oral squamous carcinoma based on GEO datasets and the Human Autophagy Database (HADb). Expressions of autophagy-related genes were further verified in Human Protein Atlas (HPA) database and GSE78060 dataset. Tumor immune estimation resource (TIMER) database was used to analyze the correlation between FADD expression and immune cell infiltration in oral carcinoma. Our findings provided reliable and potential therapeutic targets for oral carcinoma.

2 | MATERIALS AND METHODS

2.1 | Collection of gene expression profile dataset

We collected the gene expression profiles of GSE146483 and GSE23558 from the GEO database (https://www.ncbi.nlm.nih.gov/). The GSE146483 dataset contained 3 health samples and 11 oral carcinoma samples; The GSE23558 dataset was composed of 5 health samples and 27 oral carcinoma samples. The autophagy-related genes were collected from the HAMdb (http://hamdb.scbdd.com/).

2.2 | Identification of DEGs

DEGs between normal and oral carcinoma samples were further identified based on the $|\log_2 FC| > 1$ and $p < 0.05$.[17] The volcano diagram was generated using a bioinformatics online tool (http://www.bioinformatics.com.cn/). A Venn online tool was performed to obtain the intersection of upregulated or downregulated autophagy-related DEGs.

2.3 | Functional enrichment analyses of autophagy-related DEGs

Metascape (http://metascape.org/) is a gene analysis and annotation resource and was used to carry out functional enrichment analyses, including GO-BP and KEGG enrichment analyses.[18] $p < 0.05$ was considered a significant enrichment. To visualize the results of GO-BP and KEGG enrichment analyses, a bioinformatics online tool (http://www.bioinformatics.com.cn/) was used to generate the bubble diagrams.

2.4 | PPI network construction and hub genes identification

The STRING database (www.string-db.org) was used to construct the PPI network of autophagy-related DEGs. The minimum required score of 0.9 was regarded as the threshold. Hub genes were further identified with cytoHubba plugged in Cytoscape.

2.5 | The human protein atlas

Human protein atlas (HPA) is an open database that allows researchers in academia and industry free access to explore human proteome.[19] In the present study, we used the HPA database (http://www.proteinatlas.org/) to verify the protein expression of the 5 hub genes selected from normal tissues and tumor tissues by immunohistochemistry.

2.6 | Validation of FADD expression

The mRNA expression levels of FADD between tumor tissues and non-tumor tissues were verified by the GSE78060 dataset. A total of 30 samples were used, including 26 advanced tongue squamous cell carcinoma samples and 4 non-tumor samples. TCGA database was also used to verify the FADD expression in oral cancer, which included 329 tumor tissues and 32 tumor-adjacent tissues. $p < 0.05$ indicated statistical significance.

2.7 | Survival analysis of FADD in oral cancer

In the present research, the survival analysis of FADD in oral cancer was performed using a Kaplan–Meier plotter (http://kmplot.com/analysis).

2.8 | Gene set enrichment analysis

The normalized RNA-Seq data collected from the TCGA database were used to carry out Gene set enrichment analysis (GSEA).[20] In this study, the GO terms were analyzed by using GSEA to explore the possible biological function of FADD in oral cancer. P.adj <0.05 and false discovery rate (FDR) <0.25 were considered statistically significant.

2.9 | Tumor immune estimation resource database

TIMER database can be used to systematically evaluate immune cell infiltration in various types of cancers. This database was used to analyze the correlation between immune cell infiltration (dendritic cell, neutrophil, macrophage, CD4+ T cell, CD8+ T cell, and B cell) and FADD expression.

2.10 | Statistical analyses

R software was used to carry out all statistical analyses. The ROC curve was visualized by using the pROC package. Mann–Whitney U test and paired t test were used to evaluate the differential expression level of FADD between tumor samples and normal samples.

3 | RESULTS

3.1 | Identification of autophagy-related DEGs from the GEO database

Based on the screening criteria: $|\log_2 FC| > 1$ and $p < 0.05$, 6759 downregulated and 2413 upregulated DEGs were identified between the 3 health samples and 11 OSC samples from the GSE146483 dataset (Figure 1A), and 2977 downregulated and 1902 upregulated DEGs were identified between the 5 health samples and 27 OSC samples from GSE23558 dataset (Figure 1B). Subsequently, the Venn diagrams of the autophagy-related DEGs in the two datasets were mapped by a Venn online tool. As shown in Figure 2 and Table 1, a total of 49 autophagy-related DEGs including 18 upregulated (Figure 2A) and 31 downregulated (Figure 2B) DEGs were identified.

3.2 | Enrichment analyses of autophagy-related DEGs

In this study, we performed the function enrichment analyses to understand the potential functions and pathways of autophagy-related DEGs in the development of oral cancer. As shown in Figure 3A and Table 2, the results of GO-BP analysis revealed that these upregulated DEGs were associated with an apoptotic signaling pathway,

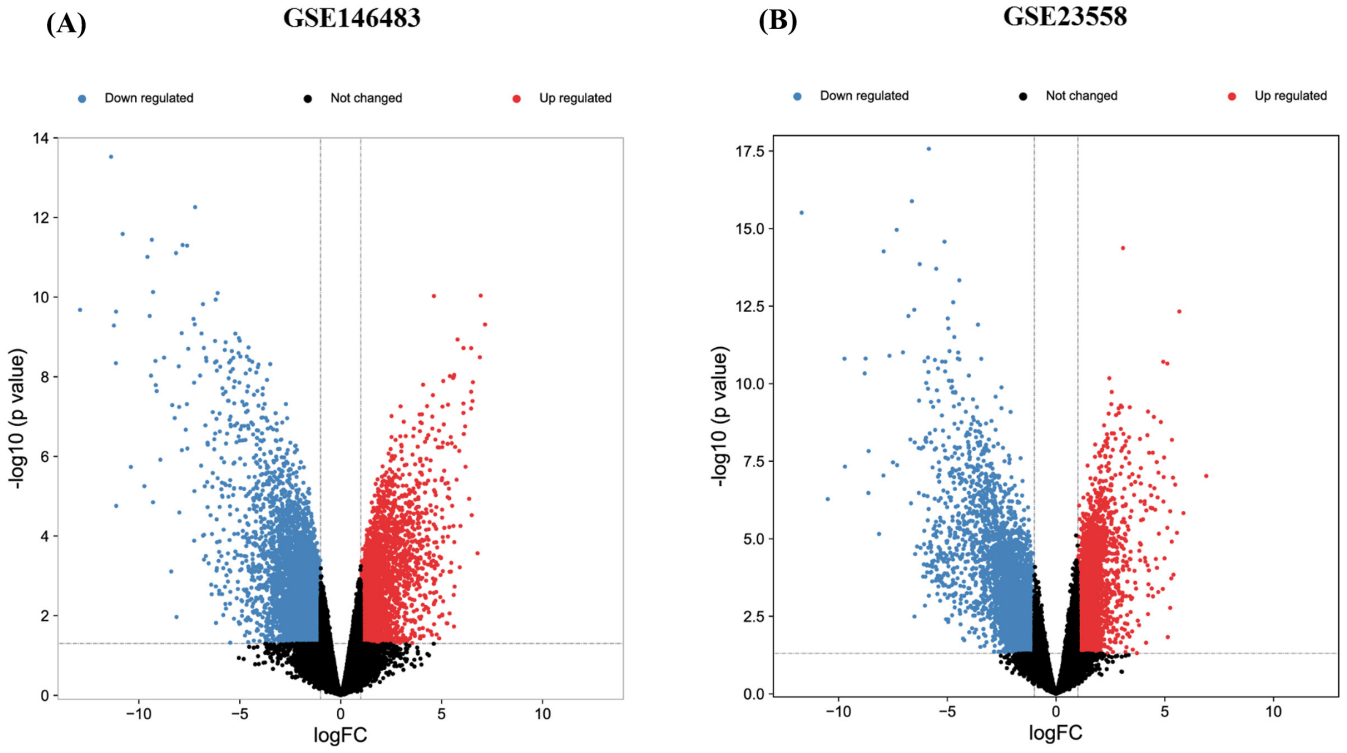


FIGURE 1 Volcano plot distribution of gene expression data between normal and OSC samples. (A) Volcano plot of GSE146483 database. (B) Volcano plot of GSE23558 database. DEGs were screened based on $|\log_2 FC| > 1$ and $p < 0.05$

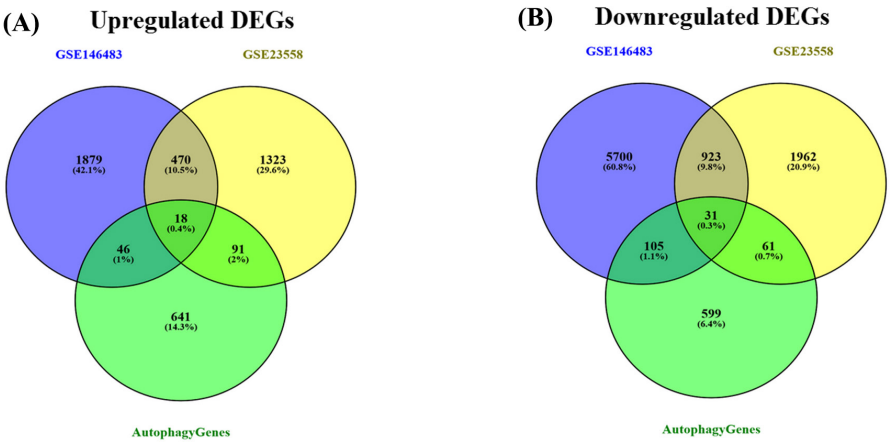


FIGURE 2 Identifying the autophagy-related DEGs among GSE146483 and GSE23558. (A) Venn diagram of the upregulated DEGs. (B) Venn diagram of the downregulated DEGs

Category	DEGs
Upregulated	EPHB2, E2F1, EIF2AK1, CHEK1, AURKA, BID, BIRC5, CSF2, TNF, ACP2, BRCA1, PTPN2, APOL6, SERPINH1, FADD, EGFR, KRT18, EIF2AK2
Downregulated	SVIP, NOS1, NUPR1, CAPNS2, CFLAR, MAP1LC3A, GAB1, TP53INP1, SESN1, ATG9B, RRAGD, DEPTOR, GJA4, LRRK2, RAB5A, TMEM74, ULK2, TBC1D9, IGF1, DCN, HTR2B, TLR7, AGT, NFE2L2, PRKAA2, GRID1, IKBKB, SYNPO2, CXCL12, PARK2, CAPN14

TABLE 1 Communal differentially expressed genes (DEGs) between GSE146483 and GSE23558 microarray data

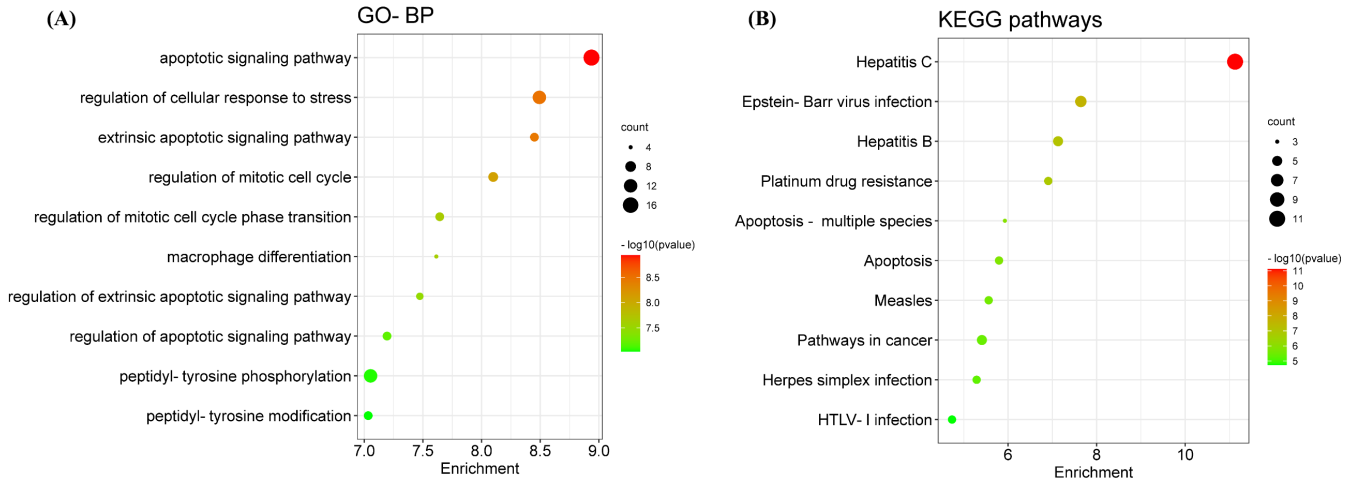


FIGURE 3 Functional enrichment analyses of upregulated DEGs. (A) The top 10 enriched GO-BP terms for upregulated DEGs. (B) The top 10 enriched KEGG pathways for upregulated DEGs

regulation of cellular response to stress, extrinsic apoptotic signaling pathway, regulation of mitotic cell cycle, and regulation of mitotic cell cycle phase transition, etc. Besides, these downregulated DEGs were significantly enriched in the process utilizing autophagic mechanism, autophagy, macroautophagy, autophagosome assembly, autophagosome organization, regulation of autophagy, etc (Figure 4A and Table 3).

Our KEGG analysis indicated that these upregulated DEGs were mainly involved in hepatitis C, Epstein–Barr virus infection, hepatitis B, platinum drug resistance, apoptosis-multiple species, apoptosis, etc (Figure 3B and Table 2). In addition, these downregulated DEGs were mainly associated with autophagy-animal, regulation of autophagy, mTOR signaling pathway, NF-kappa B signaling pathway, ras signaling pathway, longevity regulating pathway, etc (Figure 4B and Table 3).

3.3 | Construction of PPI network and identification of hub genes

STRING network-based protein interaction analysis was utilized to construct a PPI network of autophagy-related DEGs. As shown in Figure 5A, the PPI network contains 48 nodes and 25 edges. Following further analysis by Cytoscape software, these

TABLE 2 Enrichment analyses of upregulated DEGs

Category	Description	*p* value	Count	Genes
GO-BP	Apoptotic signaling pathway	1.16E−09	17	BID, BRCA1, CSF2, E2F1, KRT18, PTPN2, TNF, FADD, EIF2AK1, BIRC5, CHEK1, EGFR, AURKA, EIF2AK2, EPHB2, ACP2, APOL6
	Regulation of cellular response to stress	3.22E−09	12	BID, BRCA1, CHEK1, EGFR, EIF2AK2, PTPN2, TNF, EIF2AK1, FADD, AURKA, E2F1, KRT18
	Extrinsic apoptotic signaling pathway	3.55E−09	6	BID, BRCA1, CSF2, KRT18, TNF, FADD
	Regulation of mitotic cell cycle	7.96E−09	7	BID, BRCA1, CHEK1, E2F1, EGFR, AURKA, TNF
	Regulation of mitotic cell cycle phase transition	2.27E−08	6	BID, BRCA1, CHEK1, E2F1, EGFR, AURKA
	Macrophage differentiation	2.43E−08	4	CSF2, PTPN2, FADD, EIF2AK1
	Regulation of extrinsic apoptotic signaling pathway	3.36E−08	5	BID, BRCA1, CSF2, TNF, FADD
	Regulation of apoptotic signaling pathway	6.39E−08	6	BID, BRCA1, CSF2, PTPN2, TNF, FADD
	Peptidyl-tyrosine phosphorylation	8.83E−08	12	CSF2, EGFR, EPHB2, EIF2AK2, PTPN2, TNF, E2F1, AURKA, KRT18, FADD, BID, BRCA1
	Peptidyl-tyrosine modification	9.25E−08	6	CSF2, EGFR, EPHB2, EIF2AK2, PTPN2, TNF
KEGG	Hepatitis C	7.45E−12	11	BID, E2F1, EGFR, EIF2AK2, TNF, FADD, EIF2AK1, BIRC5, BRCA1, SERPINH1, AURKA
	Epstein–Barr virus infection	2.27E−08	6	BID, E2F1, EIF2AK2, TNF, FADD, EIF2AK1
	Hepatitis B	7.43E−08	5	BIRC5, BID, E2F1, TNF, FADD
	Platinum drug resistance	1.24E−07	4	BIRC5, BID, BRCA1, FADD
	Apoptosis-multiple species	1.18E−06	3	BIRC5, BID, FADD
	Apoptosis	1.6E−06	4	BIRC5, BID, TNF, FADD
	Measles	2.75E−06	4	BID, EIF2AK2, FADD, EIF2AK1
	Pathways in cancer	3.9E−06	5	BIRC5, BID, E2F1, EGFR, FADD
	Herpes simplex infection	5.13E−06	4	EIF2AK2, TNF, FADD, EIF2AK1
	HTLV-I infection	1.85E−05	4	CHEK1, CSF2, E2F1, TNF

autophagy-related DEGs were ranked based on their degree values, and the top 5 DEGs with the highest value, namely FADD, EGFR, TNF, IKBKB, and RRAGD, were selected as hub genes and visualized (Figure 5B). FADD was selected as a potential biomarker and further verified in the next research.

3.4 | Validation of transcriptional levels of FADD based on various databases

The TCGA database was used to assess the mRNA expression of FADD in oral cancer. As shown in Figure 6A, compared with normal samples, FADD was significantly upregulated in tumor samples ($p < 0.001$). We also used the GSE78060 dataset to verify the mRNA expression of FADD in the oral cancer tissues and normal tissues. As shown in Figure 6B, compared with normal samples, the expression of FADD was significantly upregulated in tumor samples ($p < 0.05$). Furthermore, immunohistochemical staining from the HPA database indicated that FADD protein expression was upregulated in tumor samples (Figure 6C-D). In conclusion, these findings indicated that FADD was upregulated in oral cancer patients.

3.5 | Diagnostic value of FADD for the distinction of oral cancer

In this study, a ROC curve was constructed to investigate the clinical diagnostic value of FADD in oral cancer. As shown in Figure 7A, the area under the curve (AUC) was 0.939, implying that FADD possesses diagnostic value for the distinction of oral cancer.

3.6 | Overall survival analysis of FADD

The overall survival for FADD was analyzed using a Kaplan–Meier plotter to further investigate whether FADD contributed to the overall survival in patients with oral cancer. As shown in Figure 7B,

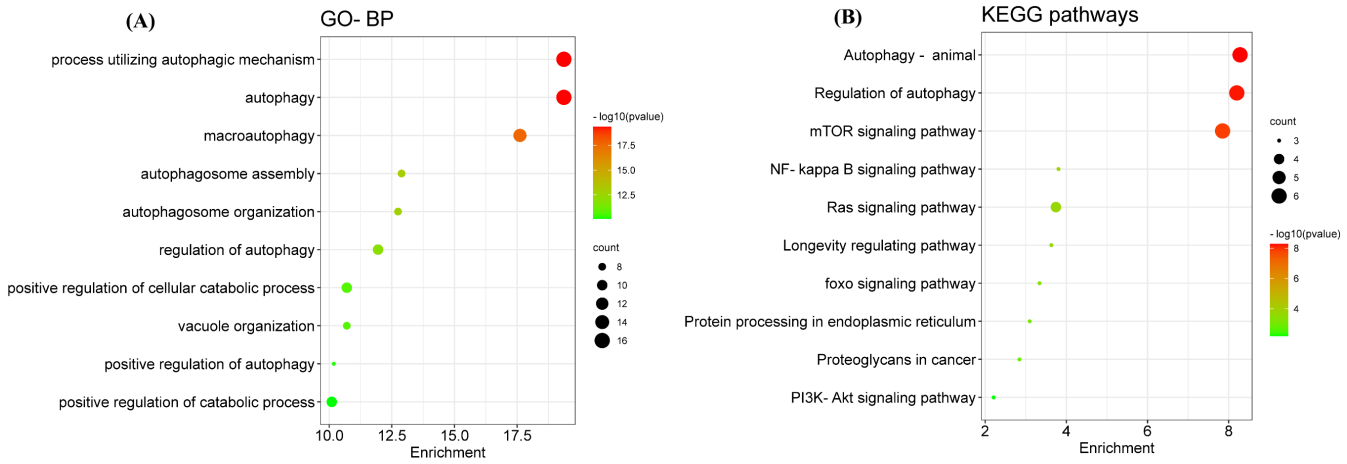


FIGURE 4 Functional enrichment analyses of downregulated DEGs. (A) The top 10 enriched GO-BP terms for downregulated DEGs. (B) The top 10 enriched KEGG pathways for downregulated DEGs

TABLE 3 Enrichment analyses of downregulated DEGs

Category	Description	*p* value	Count	Genes
GO-BP	Autophagy	4.32E−20	16	DCN, HTR2B, PRKN, PRKAA2, RAB5A, ULK2, NUPR1, SESN1, RRAGD, AP1LC3A, TP53INP1, LRRK2, TMEM74, SYNPO2, SVIP, ATG9B
	Process utilizing autophagic mechanism	4.32E−20	16	DCN, HTR2B, PRKN, PRKAA2, RAB5A, ULK2, NUPR1, SESN1, RRAGD, MAP1LC3A, TP53INP1, LRRK2, TMEM74, SYNPO2, SVIP, ATG9B
	Macroautophagy	2.45E−18	13	DCN, PRKN, PRKAA2, RAB5A, ULK2, NUPR1, SESN1, MAP1LC3A, TP53INP1, LRRK2, TMEM74, SYNPO2, ATG9B
	Autophagosome assembly	1.29E−13	8	RAB5A, ULK2, NUPR1, MAP1LC3A, TP53INP1, LRRK2, SYNPO2, ATG9B
	Autophagosome organization	1.79E−13	8	RAB5A, ULK2, NUPR1, MAP1LC3A, TP53INP1, LRRK2, SYNPO2, ATG9B
	Regulation of autophagy	1.12E−12	10	DCN, HTR2B, PRKN, PRKAA2, NUPR1, SESN1, RRAGD, TP53INP1, LRRK2, SVIP, IGF1, NFE2L2
	Positive regulation of cellular catabolic process	1.98E−11	10	DCN, IGF1, NFE2L2, PRKN, PRKAA2, NUPR1, SESN1, TP53INP1, LRRK2, SVIP
	Vacuole organization	1.98E−11	8	RAB5A, ULK2, NUPR1, MAP1LC3A, TP53INP1, LRRK2, SYNPO2, ATG9B
	positive regulation of autophagy	6.51E−11	7	DCN, PRKN, PRKAA2, SESN1, TP53INP1, LRRK2, SVIP
	Positive regulation of catabolic process	7.88E−11	10	DCN, IGF1, NFE2L2, PRKN, PRKAA2, NUPR1, SESN1, TP53INP1, LRRK2, SVIP
KEGG	Autophagy-animal	5.26E−09	6	PRKAA2, CFLAR, ULK2, RRAGD, DEPTOR, ATG9B, IGF1, IKBKB, SESN1, MAP1LC3A, TP53INP1
	Regulation of autophagy	6.33E−09	6	PRKAA2, CFLAR, ULK2, RRAGD, DEPTOR, ATG9B
	mTOR signaling pathway	1.42E−08	6	IGF1, IKBKB, PRKAA2, ULK2, RRAGD, DEPTOR
	NF-kappa B signaling pathway	0.000156	3	IKBKB, CXCL12, CFLAR
	Ras signaling pathway	0.000181	4	GAB1, IGF1, IKBKB, RAB5A
	Longevity regulating pathway	0.000234	3	IGF1, PRKAA2, SESN1
	foxo signaling pathway	0.000458	3	IGF1, IKBKB, PRKAA2
	Protein processing in endoplasmic reticulum	0.000801	3	NFE2L2, PRKN, SVIP
	Proteoglycans in cancer	0.00143	3	DCN, GAB1, IGF1
	PI3K-Akt signaling pathway	0.006205	3	IGF1, IKBKB, PRKAA2

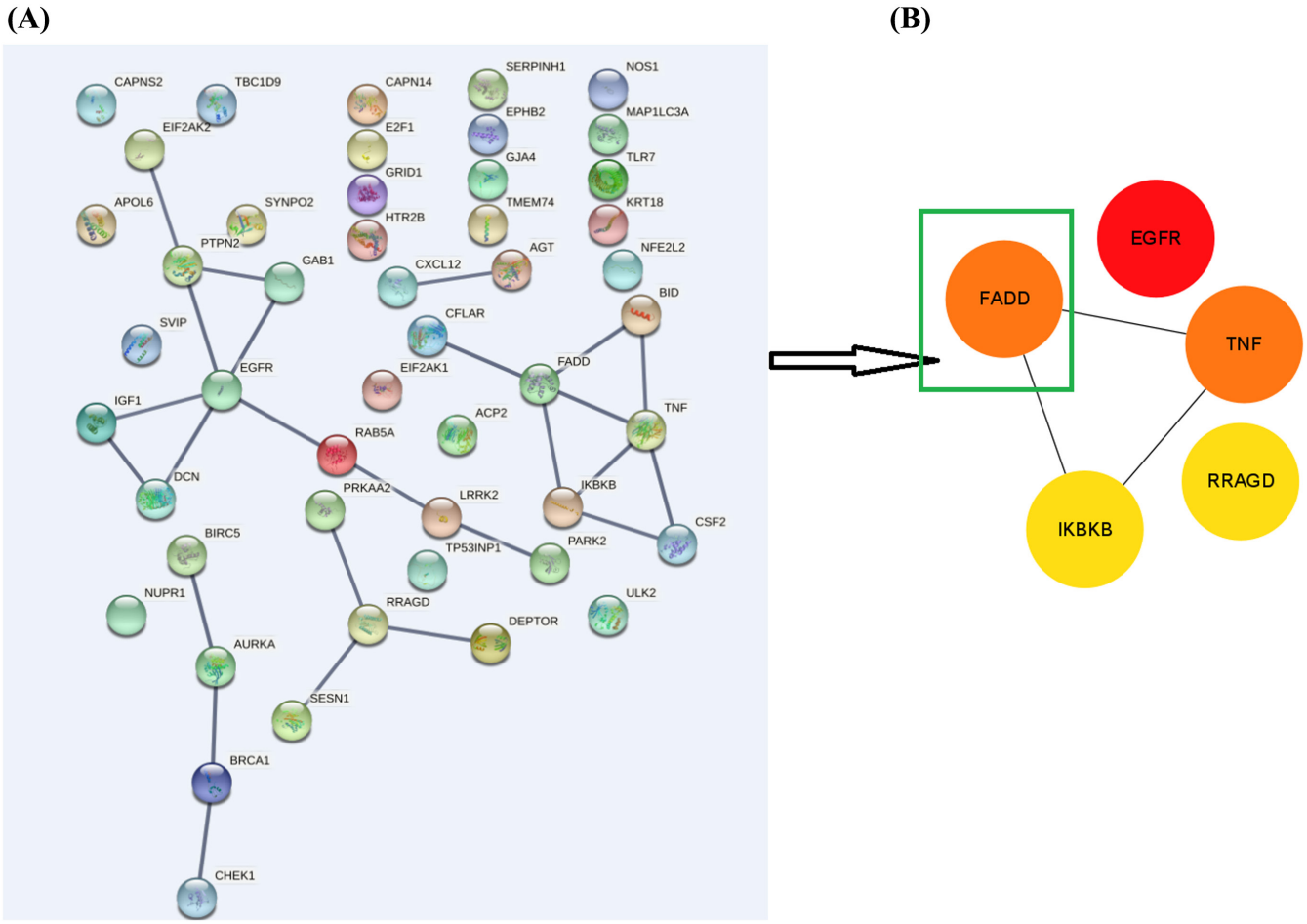
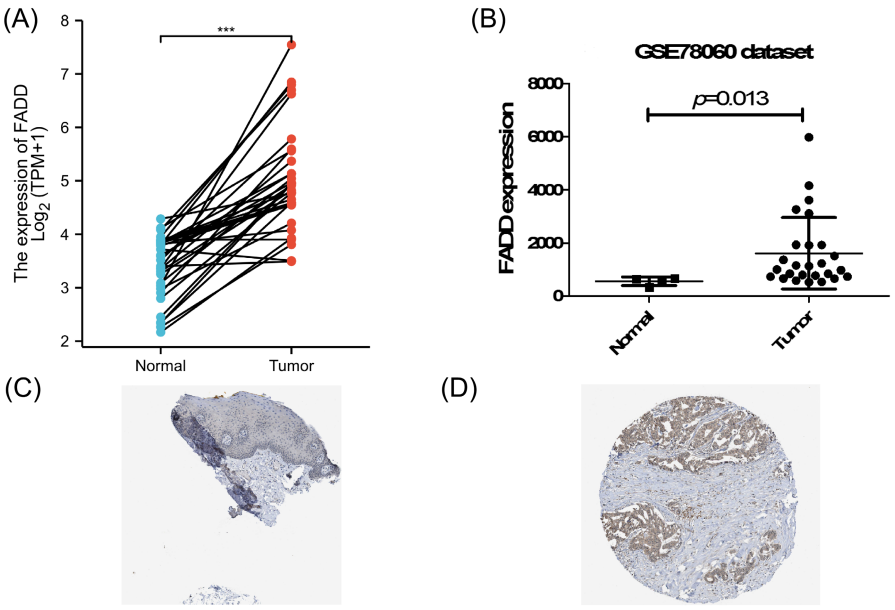


FIGURE 5 Identification of hub genes. (A) PPI network of the overlapping DEGs. (B) The top 5 hub genes were identified based on the degree of nodes

FIGURE 6 Expression level of FADD in oral cancer. (A) The mRNA expression level of Fais DD based on TCGA the database. (B) The mRNA expression level of FADD is based on the GSE78060 dataset. (C) Protein levels of FADD in normal oral tissue (staining: not detected; intensity: negative; and quantity: none). (D) Protein levels of FADD in tumor tissue (staining: medium; intensity: moderate; and quantity: >75%)



our findings indicated that the high expression of FADD level was related to the worse overall survival in patients with oral cancer, which demonstrated that FADD was associated with oral squamous carcinoma progression and may be used as a tumor biomarker for oral cancer sufferers.

3.7 | GSEA identifies FADD-associated pathways in oral cancer

In the present study, we performed GSEA analysis based on the TCGA database to evaluate the potential function of FADD in

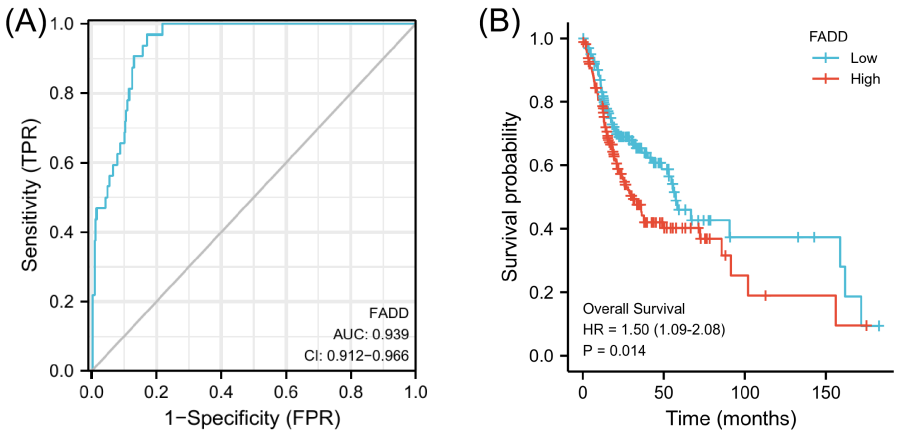


FIGURE 7 ROC curve and overall survival curve for FADD in oral cancer. (A) ROC curve revealed that FADD exhibited an AUC value of 0.939 to distinguish between normal samples and oral cancer samples. (B) Higher FADD expression resulted in shorter overall survival

the progression of oral cancer. The GSEA indicated that FADD-associated genes mainly enriched in immune-related pathways, such as positive regulation of immune response (NES = −2.324, p.adj = 0.045, FDR = 0.039; Figure 8A), lymphocyte activation (NES = −1.946, p.adj = 0.045, FDR = 0.039; Figure 8B), leukocyte-mediated immunity (NES = −2.064, p.adj = 0.045, FDR = 0.039; Figure 8C), innate immune response (NES = −1.793, p.adj = 0.045, FDR = 0.039; Figure 8D), and adaptive immune response (NES = −2.786, p.adj = 0.045, FDR = 0.039; Figure 8F). Besides, the endocytosis (NES = −1.984, p.adj = 0.045, FDR = 0.039; Figure 8E) was significantly negatively correlated with FADD expression. These results implied that immune-related pathways were involved in the progression of oral cancer. Therefore, we further investigated the correlation between FADD expression and immune infiltrates in oral cancer.

3.8 | Tumor-infiltrating immune cells were associated with FADD expression in oral cancer

In this research, we further assessed the correlation between immune infiltration levels and FADD expression in oral cancer by using the TIMER database. As shown in Figure 9A, our results revealed that FADD expression exhibited a significantly positive correlation with infiltrating levels of Macrophages ($p = 9.46 \times 10^{-4}$), and CD4 + T cells ($p = 4.59 \times 10^{-2}$), and a significantly negative correlation with infiltrating levels of B cells ($p = 8.15 \times 10^{-3}$). Besides, we further analyzed the immune infiltration levels in HNSC with copy number variations of FADD. As shown in Figure 9B, the copy number variations of FADD were significantly correlated with infiltrating levels, such as dendritic cells, neutrophil, CD4 + T cells, CD8 + T cells, and B cells. In the present study, the differences in tumor-infiltrating immune cells between the FADD high-expression and FADD low-expression groups were also compared to evaluate the role of the tumor immune microenvironment in oral cancer. Our results showed that the expression of T cells, Th2 cells, Th17 cells, TFH, mast cells, eosinophils, DC, cytotoxic cells, and B cells was significantly different between FADD low-expression and FADD high-expression groups (Figure 9C). These results revealed that FADD plays an important role in the immune infiltration of oral cancer.

4 | DISCUSSION

Oral cancer is a progressive disease with a high mortality rate and unfavorable prognosis. Although the advancements in chemotherapy, radiation, and surgery for the treatment of oral cancer, the etiopathogenesis of oral cancer is not fully understood and five-year survival rates for oral cancer sufferers have not improved.[21] Therefore, to improve individualized treatment and prognosis evaluation, it is critically necessary to further identify potential molecular biomarkers and therapeutic targets for early diagnosis, prevention, and treatment of oral cancer.[22] Autophagy is a conserved lysosomal degradation process that involves metabolic adaptation and nutrient cycling and plays an important role in the pathogenesis of cancers. Previous studies have demonstrated that the development and progression of oral cancer are closely associated with autophagy impairment.[23,24] Thus, the present study aimed to identify autophagy-related genes and assess immune cell infiltration based on bioinformatics approaches.

In the present study, we first collected all genes from the GEO database. Then, 49 autophagy-related DEGs were identified in tumor samples, including 18 upregulated genes and 31 downregulated genes. Subsequently, we performed GO and KEGG enrichment analyses to investigate the potential molecular mechanisms of autophagy-related DEGs. Our findings indicated that the autophagy-related DEGs were mainly enriched in the regulation of the following pathways: apoptotic signaling pathway, regulation of cellular response to stress, platinum drug resistance, a process utilizing autophagic mechanism, autophagy, regulation of autophagy, mTOR signaling pathway, NF-kappa B signaling pathway, Ras signaling pathway, and longevity regulating pathway, etc. These key pathways are potentially related to autophagy or the etiopathogenesis of oral cancer. Previous studies have demonstrated that the oncoapoptotic signaling pathway and their downstream pathways play an important role in oral cancer development.[25-27]

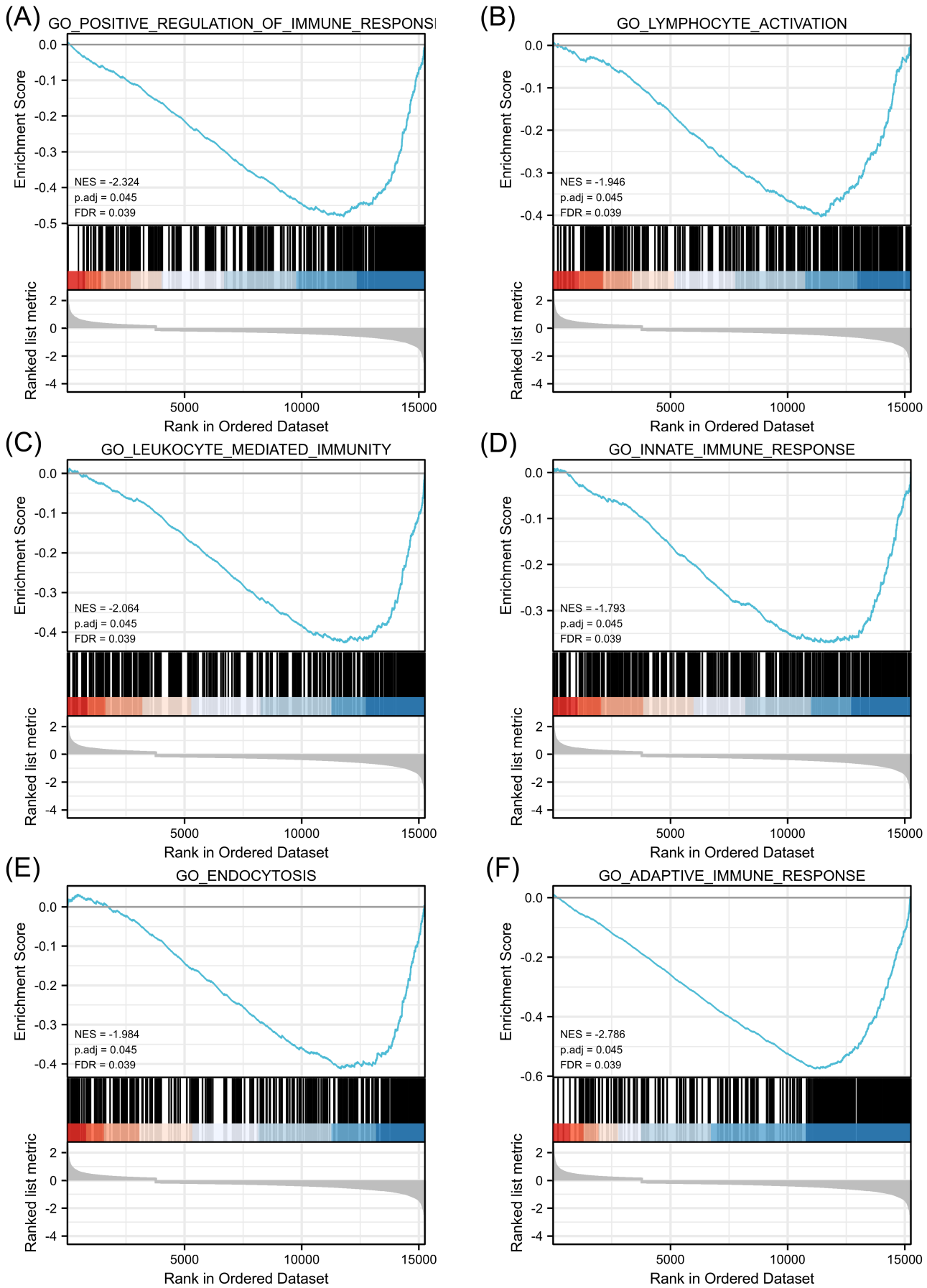


FIGURE 8 Enrichment plots of GSEA. The findings revealed that the positive regulation of immune response (A), lymphocyte activation (B), leukocyte-mediated immunity (C), innate immune response (D), endocytosis (E), and adaptive immune response (F) were significantly enriched in oral cancer samples with high FADD expression

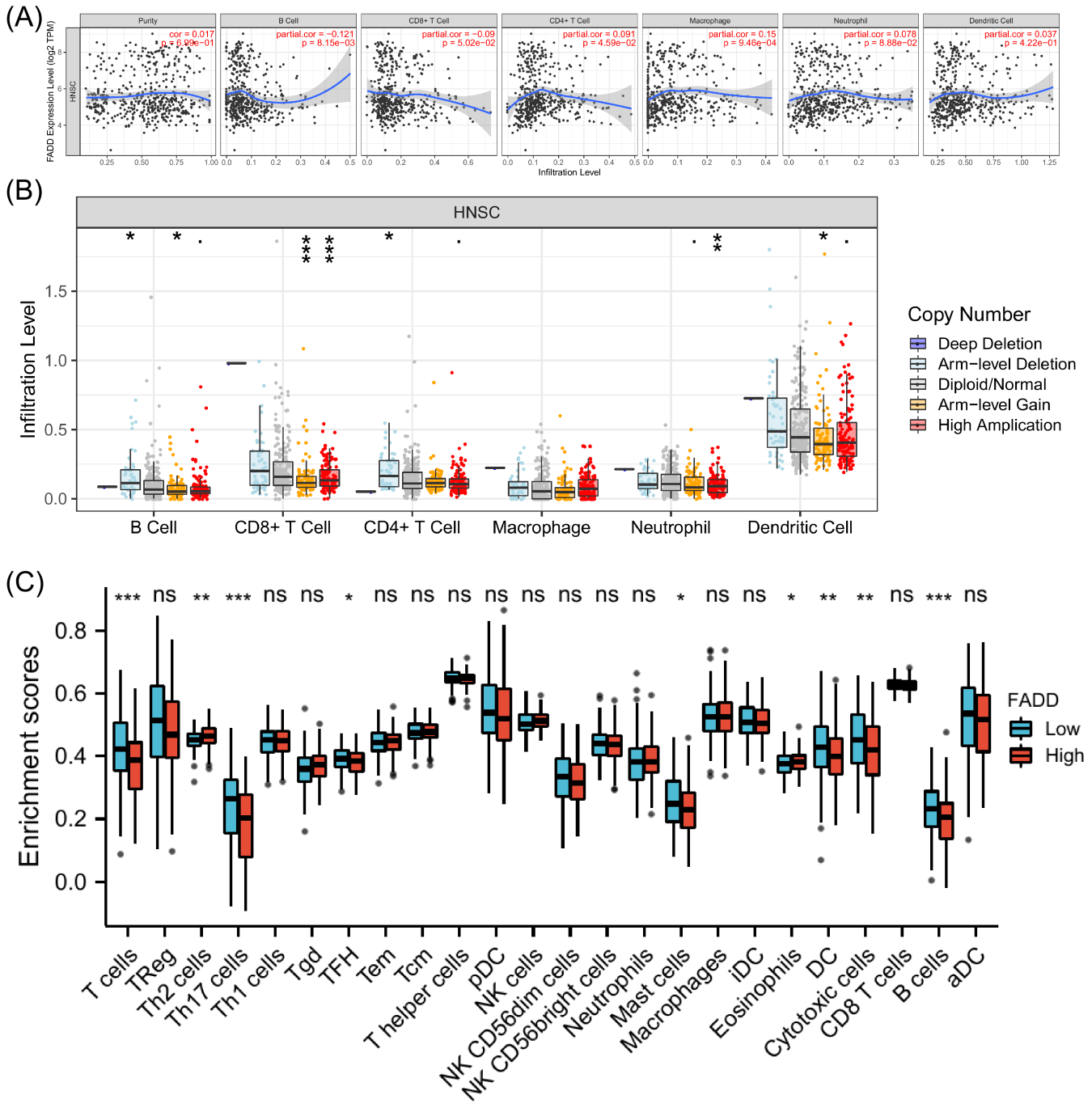


FIGURE 9 FADD expression is related to immune cell infiltrations in oral cancer. (A) Correlation analysis between FADD expression and immune cells. (B) The copy number variations of FADD affect the infiltrating levels of dendritic cells, neutrophil, macrophages, CD4 + T cells, CD8 + T cells, and B cells. (C) The differential expression of tumor-infiltrating immune cells in high and low FADD expression groups (ns: no significance, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$)

For example, abnormal expression of apoptosis pathway proteins in neutrophils of patients with oral cancer[28] and targeting apoptosis-related pathways in the oral cancer cells might be a potential therapeutic strategy for oral cancer.[29,30] Platinum drug resistance plays a vital role in oral squamous cell carcinoma therapy.[31] The activated mTOR signaling pathway has been involved in the carcinogenesis of oral cancer and mTOR inhibitors are considered as promising candidates for the treatment of oral cancer.[32,33] For example, gingerol inhibits oral cancer cell growth via the inactivation of the AKT/mTOR signaling pathway.[34] Activation of the NF-kappa B signaling pathway is involved in oral tumorigenesis.[35] In addition, NF-kappa B plays an important role in the progression of both radiation and chemoresistance in head and neck cancer, which is considered as a major cause for the failure of therapy.[36] Targeting NF-kappa B might be a novel effective therapy against oral cancer.[37,38] Therefore, both GO and KEGG enrichment

analyses revealed these autophagy-related DEGs are relevant to oral cancer progression.

Subsequently, we constructed a PPI network and identified 5 hub genes, including EGFR, FADD, TNF, IKBKB, and RRAGD. Further, the results of immunohistochemistry and mRNA analysis verified that the expression levels of FADD were higher in the oral cancer tissues than that in the non-tumor tissues. Fas-associated death domain (FADD), a key adaptor protein, transmits apoptotic signals regulated by death receptors, TNF-R1, FAS, and other molecules, thereby promoting caspase activation.[39] FADD has been indicated to regulate MAPK and NF-kappa B signaling pathways, which in turn could promote cell cycle, inflammation, innate immunity, and cancer progression.[40] Overexpression of FADD in head and neck cancer patients was related to worse survival and shorter survival times.[41] FADD gene might be considered a potential prognostic marker and is related to lymph node metastasis in oral cavity squamous cell carcinomas sufferers.[42] In the present study, GSEA indicated that FADD-associated genes were mainly enriched in immune-related pathways, such as positive regulation of immune response, lymphocyte activation, leukocyte-mediated immunity, and innate immune response. Therefore, we speculated that the upregulation of FADD expression might influence the tumor immune microenvironment in oral cancer.

Previous reports have demonstrated the close correlations between immunity and autophagy.[43,44] Recent research has revealed that autophagy could command immune responses via regulating the secretion of cytokines and the functions of immune cells.[45,46] CD4+T cells have high immunosuppressive effects and promote tumor progression via inhibiting effective antitumor immunity.[47] B cells also have been indicated to play an important role in modulating immune responses involved in tumor, autoimmunity, and inflammation.[48] Tumor-related macrophages promoted tumor progression via taming protective adaptive immunity, nurturing cancer stem cells, and promoting genetic instability.[49] In this research, we used the TIMER database to explore the correlation between FADD expression and immune infiltration in oral cancer. Our findings indicated that FADD expression was significantly associated with macrophages, CD4+T cells, and B cells. Besides, it also demonstrated that the copy number variations of FADD were significantly correlated with infiltrating levels, such as dendritic cells, neutrophil, CD4 + T cells, CD8 + T cells, and B cells. Our results also indicated that the expression of T cells, Th2 cells, Th17 cells, TFH, mast cells, eosinophils, DC, cytotoxic cells, and B cells was significantly different between FADD low-expression and FADD high-expression groups. These results implied that FADD plays a vital role in the recruitment and modulation of immune cell infiltration in oral cancer. However, whether FADD could become the therapeutic target still needs a further pre-clinical and clinical trial.

In summary, we demonstrated that FADD expression was upregulated and associated with a poor prognosis in oral cancer. Besides, FADD expression was also associated with various immune cells and might influence oral cancer tumor immunity via suppressing the B cells infiltration. FADD may be regarded as a potential biomarker in patients with oral cancer.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT

All data involved in the present study are available from the corresponding author on reasonable request.

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