Original Article

Identification of Potential Molecular Mechanisms and Prognostic Markers for Oral Squamous Cell Carcinoma: A Bioinformatics Analysis

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ABSTRACT

Aims and Objectives: The goal of this study was to uncover crucial biochemical pathways, prognostic indicators, and therapeutic targets in patients with oral cancer in order to enhance therapy strategies. **Materials and Methods:** Five gene expression omnibus datasets were analyzed by using bioinformatics approaches to identify differentially expressed genes (DEGs). To determine biological alterations, gene ontology (GO) and KEGG pathway analyses were implied using the identified DEGs. Hub genes were determined using protein–protein interaction (PPI) network analysis and an interactome was constructed using NetworkAnalyst. Furthermore, five hub genes were evaluated for use as prognostic markers by using the human protein atlas (HPA) and the GEPIA2.0 database. In addition, the correlations between hub-gene expression and immune cell infiltration of oral squamous cell carcinoma (OSCC) tumors were analyzed using the tumor immune estimation resource (TIMER) database. **Results:** A total of 2071 upregulated genes and 1893 downregulated genes were identified. GO and pathway analysis showed DEGs were enriched in multiple immune response terms and interaction of inflammatory cytokines. From the PPI network, five hub genes were identified that have a crucial role in OSCC. These included interferon regulatory factor 4 (IRF4), chemokine receptor 7 (CCR7), TNF receptor superfamily member 17 (TNFRSF17), CD27, and sphingosine-1-phosphate receptor 4 (S1PR4), which were predicted to be favorable prognostic markers for OSCC using HPA. Overall survival analysis revealed that low expression of the five hub genes was significantly associated with worse overall survival. Our analysis of tumor-associated immune infiltration revealed that increased IRF4 expression was positively correlated with the gene expression profiles suggestive of infiltration of all immune cell types, whereas increased CCR7 expression was negatively correlated with neutrophil infiltration. Increased expression of CD27, S1PR4, and TNFRSF17 was found to be negatively correlated with dendritic cell, M0 macrophage, and neutrophil infiltration. **Conclusion:** In summary, inflammation, and the immune response play an important role in OSCC. All five hub genes were good predictors of OSCC prognosis, suggesting that they could be used as potential therapeutic targets and tumor markers.

Keywords: *Bioinformatics analysis, immune response, inflammation, oral squamous cell carcinoma, prognostic marker*

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Introduction

One of the most prevalent head and neck squamous cell carcinomas (HNSCC) is oral squamous cell carcinoma (OSCC), which accounts for more than 200,000 new cancer cases per year globally.[1] OSCC differs from other malignancies due to its invasive development, frequent regional metastases, high recurrence rate, and poor prognosis.[2] OSCC has a complex etiology. It has been observed that cigarette smoking, excessive alcohol intake, and betel quid use are the key causes and common etiological factors in OSCC.[3] Although the oral examination is simple, many patients go misdiagnosed until they are in the advanced clinical stages, resulting in a dismal prognosis and a significant risk of death.[4] Despite the availability of effective treatments such surgical resection, chemotherapy, radiation, and targeted biologics, the 5-year overall survival rate for patients with OSCC remains low. Therefore, early diagnosis is the most critical factor in enhancing the 5-year survival rate and prognosis of OSCC.[5]

Recent years have seen an increase in OSCC cases, particularly among young persons.[6] Therefore, additional research is required to comprehend the molecular causes of this disease. Recent research has shown that the development and growth of OSCC tumors are correlated with distinctive changes in gene expression levels.[7] Finding plausible processes and mRNA biomarkers for OSCC risk assessment, treatment, and early diagnosis is still urgently needed.[8]

At present, the fields of bioinformatics and computer science are very important to the study of biology, especially for the analysis of protein and gene regulatory networks. They aimed at identifying key genes and proteins involved in the pathogenesis of different diseases.[9] Bioinformatics allows researchers to organize big data into a more manageable form that facilitates access and the addition of new features as they become obtainable. It also makes possible the development of resources and instruments that aid researchers analyze and interpret data in an attempt to provide a biological explanation for these observations.[10] Bioinformatics enables access to various forms of data, such as sequencing and expression results on a large scale, enabling whole-genome and whole-exome investigations.[11]

This work provides a novel perspective for understanding OSCC development's essential molecular mechanism, which could offer more promising possible biomarkers for early detection, prognosis, and treatment.

Materials and Methods

Gene expression omnibus dataset and selection

The following criteria were used to search the gene expression omnibus (GEO) database: Search term, "Oral Squamous Cell Carcinoma"; study type, "Expression profiling by array"; publication dates, 2010/1/1-2018/11/21. Four datasets, GSE37991, GSE30784, GSE23558, GSE56532, and GSE74530 from Taiwan, USA, India, and Australia, respectively, were incorporated into the current analysis. The flowchart is shown in Figure 1 and Table 1. The datasets included 320 OSCC samples and 104 normal oral mucosa samples. The use of five gene expression profiles averted any confounding due to differences based on sample heterogeneity within single profiles and revealed universal differentially expressed genes (DEGs) that apply to different ethnic groups. It has been reported that ethnic differences may affect disease-associated gene expression profiles.[11] The raw data were applied for Imageo (http://bioinfo.genyo.es/imageo/) and screened with the limma package in R programming (version 3.2.5; http://www.r-project.org/). After that, the DEGs between OSCC and normal oral mucosa tissues were screened using the $\log_2$ of the fold change (logFC). An adjusted *P*-value <0.05 and |logFC|>2 were defined as the cutoff standards.

Biological function and pathway analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID; http://david.ncifcrf.gov/home.jsp) is a bioinformatics database that combines biological data mining and technologies to give systematic, integrated biometric annotation information for huge lists of genes or proteins.[12] DAVID was used to conduct gene ontology (GO) analysis and KEGG pathway enrichment on DEGs in order to identify the GO terms in the categories: biological process, cellular component (CC), molecular function (MF), and the signaling transductions of the participating DEGs. A value of $P < 0.05$ was considered statistically significant.

Protein network construction and identification of candidate genes

Through the quick construction of biological networks, NetworkAnalyst (https://www.networkanalyst.ca) is a suite of web-based online tools for statistical meta-analysis, data integration, and data visualization. It supports the meta-analysis of gene lists and integrates data using robust statistical methods, which are then visually examined within protein–protein interaction (PPI) networks.[13] In this investigation, STRING was

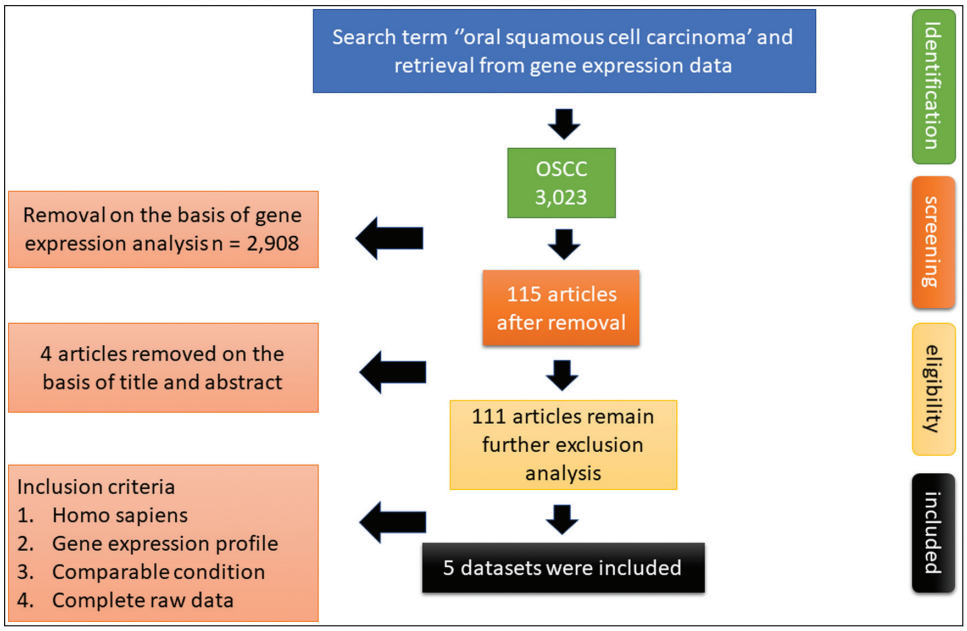


Figure 1: Flowchart of study selection in bioinformatics analysis process

Table 1: Number of normal and disease samples in the five gene expression omnibus datasets

Dataset	Normal	Disease
GSE74530	6	6
GSE34106	15	28
GSE34105	16	62
GSE25099	22	57
GSE30784	45	167

chosen as a PPI database. In order to get more objective and reliable results, this study confined the sources requiring experimental proof, and the criteria cutoff score was set at high confidence (900). The nodes with the most degrees were designated as hub genes, and they may serve as core proteins or major candidates with crucial physiological regulatory roles. In the next step, genes in the modules were analyzed for pathway enrichment. A statistically significant difference was determined as $P < 0.05$.

Verification of hub- genes in The Cancer Genome Atlas cohort using the GEPIA database

To confirm the validity of all five hub genes, we examined the levels of expression in both normal tissues and tumors. Each hub gene's expression level in tumor and normal tissue was represented as a box plot. The online GEPIA2 database was used to analyze publicly available data from The Cancer Genome Atlas (TCGA) cohort to determine the relationship between hub-gene expression levels and disease-free survival in patients with HNSCC.[14] In this analysis, only patients with completed follow-up periods were chosen for survival analysis, and they were then divided into two groups based on the median hub-gene expression values. Hub genes involved in survival were considered significant if their log-rank $P < 0.05$.

Tumor immune estimation resource database

The tumor immune estimation resource (TIMER) database, which is publicly available at http://cistrome.org/TIMER, is used to assess the correlation between cancer and immune cell infiltration.[15,16] In order to identify hub genes from PPI networks, the effect of immune stimuli on OSCC and gene expression related to immune infiltration were evaluated using TIMER. Log-rank *P*-value and hazard ratio (HR) with a 95% confidence interval (CI) were used to assess statistical significance. The positive correlation was set as $P < 0.05$, Spearman's $\rho < 0$, and the negative correlation was set as $P < 0.05$, Spearman's $\rho > 0.05$.

Results

Differentially expressed gene identification

The data were screened by using the limma package with cutoff criteria set at $P < 0.05$ and $|\log FC| > 2$. The expression profile data allowed for the identification of 3964 DEGs, including 2071 upregulated and 1893 downregulated genes. Table 2 shows the findings of analyses contrasting OSCC and normal tissue expression profiles from the five datasets (GSE37991, GSE30784, GSE23558, GSE56532, and GSE74530). Figure 2A shows a heat map of the DEGs' general clustering. Figure 2B–F shows the volcano plots.

Biological function enrichment analysis

According to Figure 3A–F, the GO enrichment analysis showed that in the biological processes category (BP), the upregulated genes were significantly enriched in immune response, immune system process, and regulation of immune system process, whereas downregulated genes were enriched in oxidation–reduction process, fatty acid metabolic process, and small-molecule metabolic process. In addition, the CC revealed that the chromosome category was enriched in upregulated genes, whereas the mitochondrial protein complex category was mainly enriched in downregulated genes. In addition, for MF, upregulated genes were enriched in the macromolecular complex binding category, and downregulated genes were enriched in the oxidoreductase activity category. As shown in Figure 4A, KEGG pathway enrichment analysis indicated that the cytokine–cytokine receptor interaction, focal adhesion, and extracellular matrix (ECM)–receptor interaction pathways were enriched in OSCC-regulated genes.

Table 2: Differentially expressed genes of five datasets and meta-analysis

Dataset	Significant genes
GSE74530	1711
GSE34106	2314
GSE34105	3725
GSE25099	8401
GSE30784	7571
Meta-analysis	3964

Protein–protein interaction network analysis and hub gene identification

The STRING database was used to determine the PPI network among the 3964 DEGs. As revealed in Figure 4B–F, five hub genes were specified based on their connectivity degree from five subnetworks. The results revealed that interferon regulatory factor 4 (*IRF4*), chemokine receptor 7 (*CCR7*), TNF receptor superfamily member 17 (*TNFRSF17*), CD27 molecule (*CD27*), and sphingosine-1-phosphate receptor 4 (*S1PR4*) were the most crucial genes with the highest connectivity.

Verification in The Cancer Genome Atlas cohort by GEPIA database

After the five hub genes were identified, we verified their expression level in the patients of the TCGA cohort as shown in Figure 5A. All of the hub genes were found to be significantly upregulated in HNSCC compared with normal tissues. Moreover, overall survival analysis of all selected hub genes was performed by using the

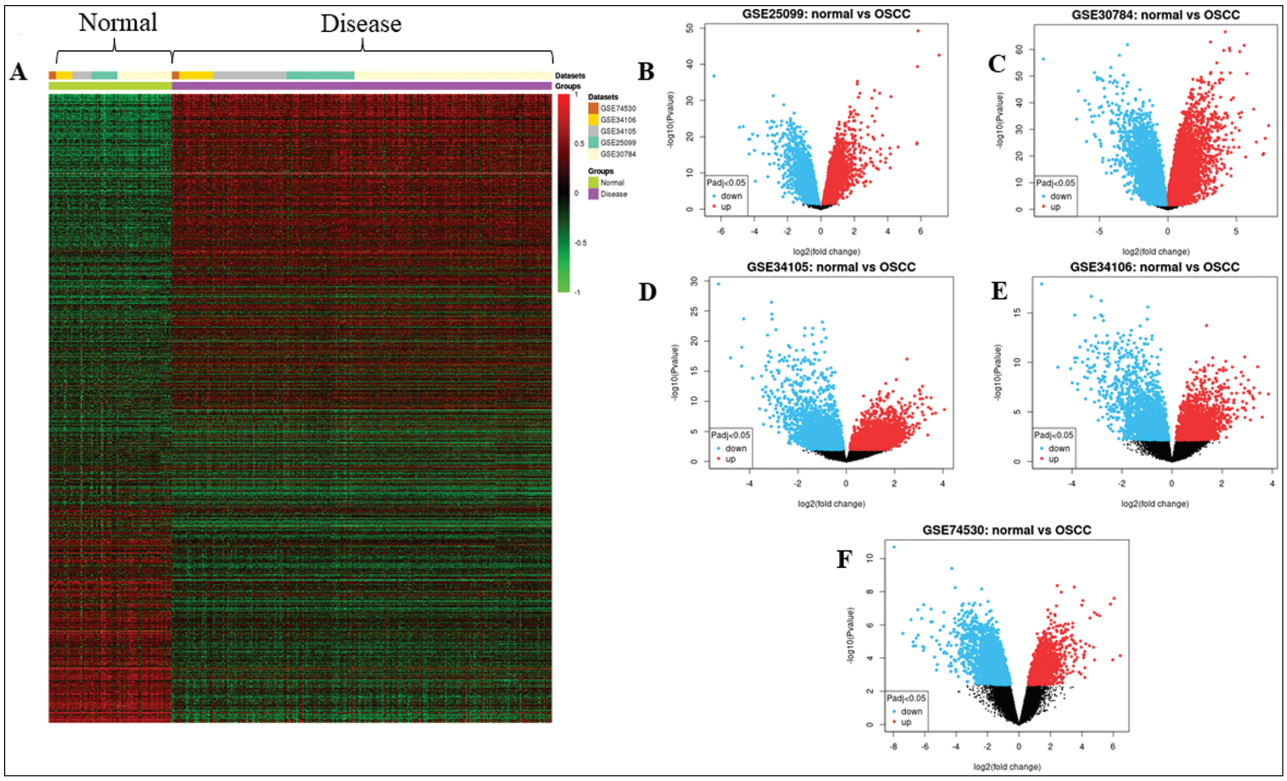


Figure 2: (A) Heatmap of overall DEGs. The red color indicates low and the green color indicates high levels of gene expression. (B–F) Volcano plots of the differentially expressed genes (DEGs) in GSE25099, GSE30784, GSE34106, GSE34105, and GSE74530. The red color represents upregulated genes ($P < 0.05$) and the blue color represents downregulated genes ($P < 0.05$)

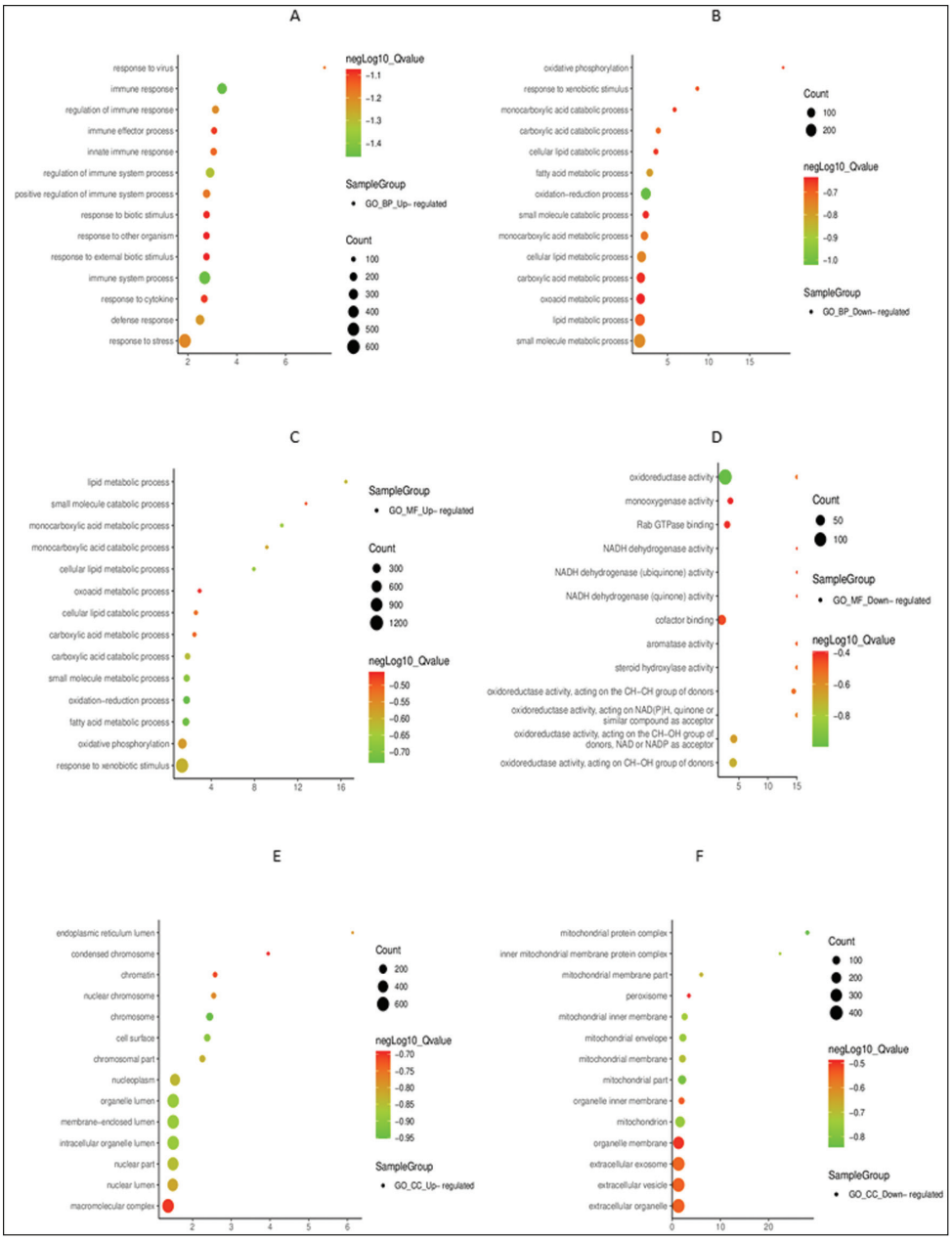
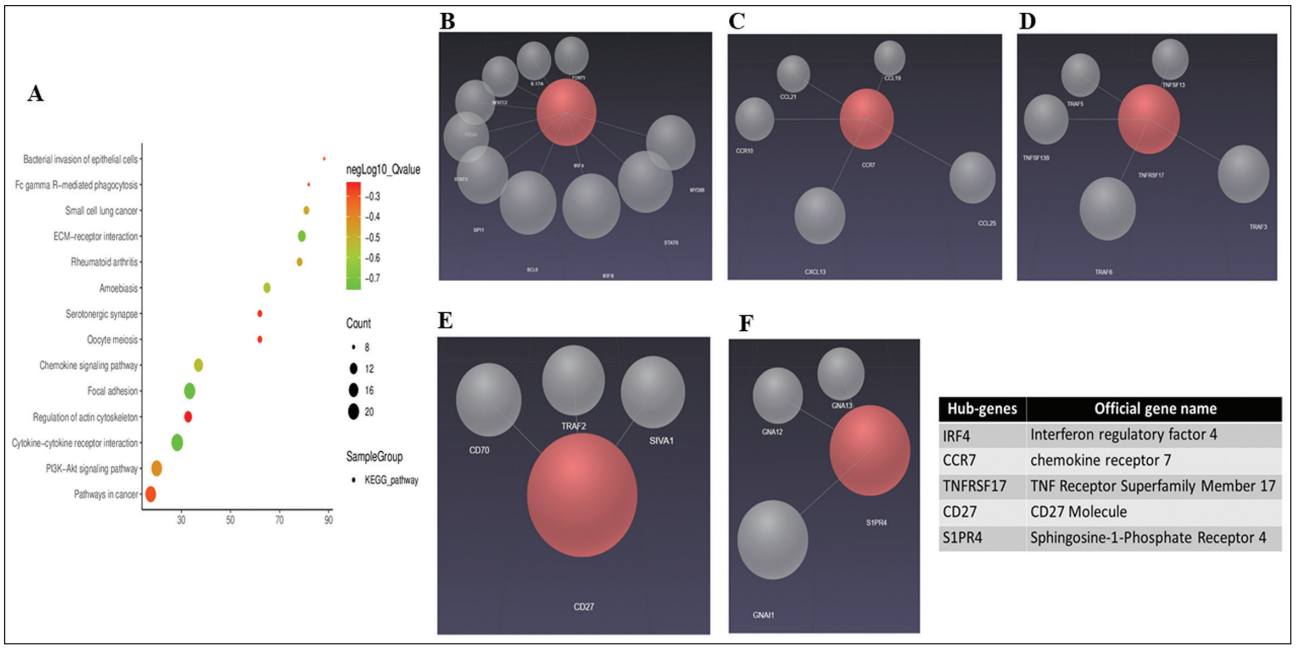


Figure 3: Terms of gene ontology (GO) analysis relating to upregulated and downregulated differentially expressed genes (DEGs). (A and B) The up- and downregulated GO terms of biological process (BP). (C and D) The up- and downregulated GO terms of molecular function (MF). (E and F) The up- and downregulated of cellular component (CC)

GEPIA2 database for validating the prognostic values of the hub genes in patients with HNSCC. The results show that lower expression of all five hub genes was significantly associated with worse overall survival in patients with HNSCC ($P < 0.05$) as shown in Figure 5B–F.

Analysis of tumor-associated immune infiltration

Immune infiltrating cells disrupt cytokine signaling in the tumor microenvironment and are a major contributor to the growth of cancer. In this study, we determined the relationship between hub gene expression and the expression profiles of immune cells, such as CD8+ T cells, CD4+ T cells, B cells, neutrophils, dendritic cells, macrophages, and natural killer (NK) cells. Our analysis showed that CCR7 was negatively connected with just neutrophils, but IRF4 was favorably correlated ($P = 0.05$) with gene expression profiles indicating infiltration by all immune cell types. Dendritic cells, M0 macrophages, and neutrophils were observed to negatively correlate with CD27, S1PR4, and TNFRSF17. Figure 6A–E shows a dot



Hub-genes	Official gene name
IRF4	Interferon regulatory factor 4
CCR7	chemokine receptor 7
TNFRSF17	TNF Receptor Superfamily Member 17
CD27	CD27 Molecule
S1PR4	Sphingosine-1-Phosphate Receptor 4

Figure 4: (A) Terms of KEGG pathway analysis. The DEGs were mainly associated with cytokine–cytokine receptor interaction, focal adhesion, and ECM–receptor interaction pathways. (B–F) The constructed protein–protein interaction networks for the hub genes: CCR7, CD27, IRF4, S1PR4, and TNFRSF17

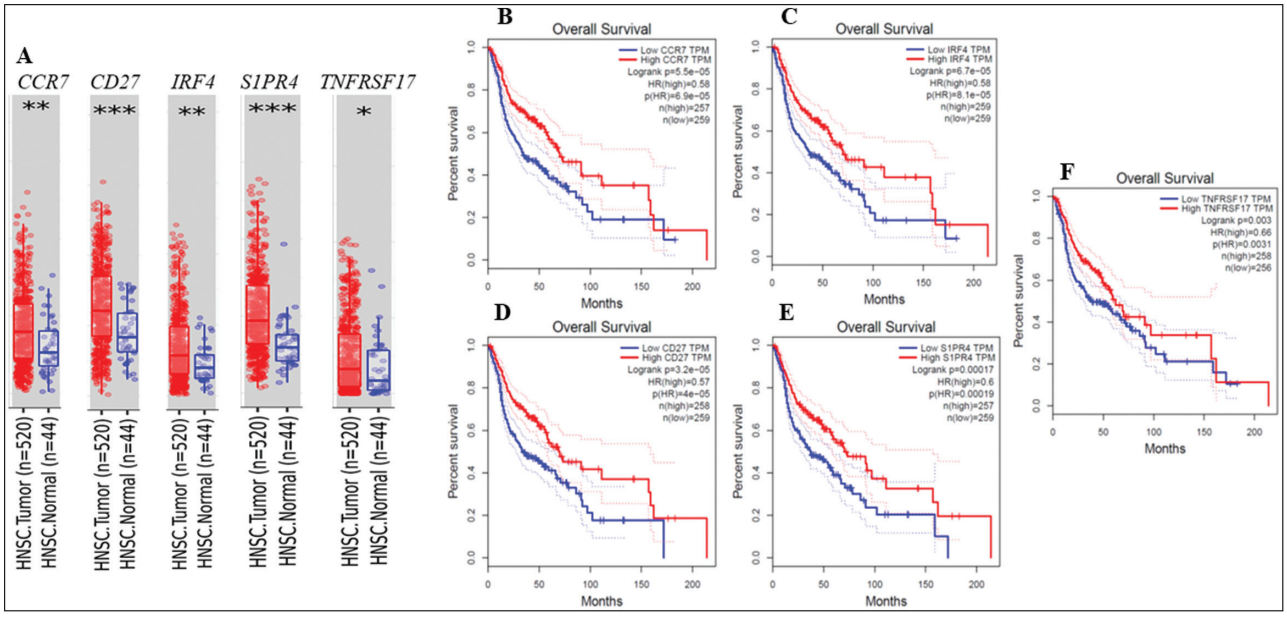


Figure 5: (A) Validation of hub-gene expression level between normal and head and neck cancer samples based on TCGA database GTEx data in GEPIA database. Overall survival analysis values of CCR7 (B), IRF4 (C), CD27 (D), S1PR4 (E), and TNFRSF17 (F) were performed by using GEPIA2.0 and Kaplan–Meier plotter online database. The solid lines indicate the survival curve and dotted lines indicate the 95% confidence interval. Log-rank $P < 0.05$ was considered to indicate a statistically significant difference. Patients with gene expression levels above the median are represented by red and blue lines

plot of associations between hub genes and immune infiltrating cells.

Discussion

Our results revealed that *IRF4*, *CCR7*, *TNFRSF17*, *CD27*, and *S1PR4* were the most potential genes in OSCC pathogenesis, which have a positive correlation with CD4+, CD8+, B cell, and NK cell in immune infiltration of tumor analysis. The overall survival analysis showed that all five hub genes were significantly upregulated in HNSCC compared with normal tissues. Currently, the incidence of OSCC is

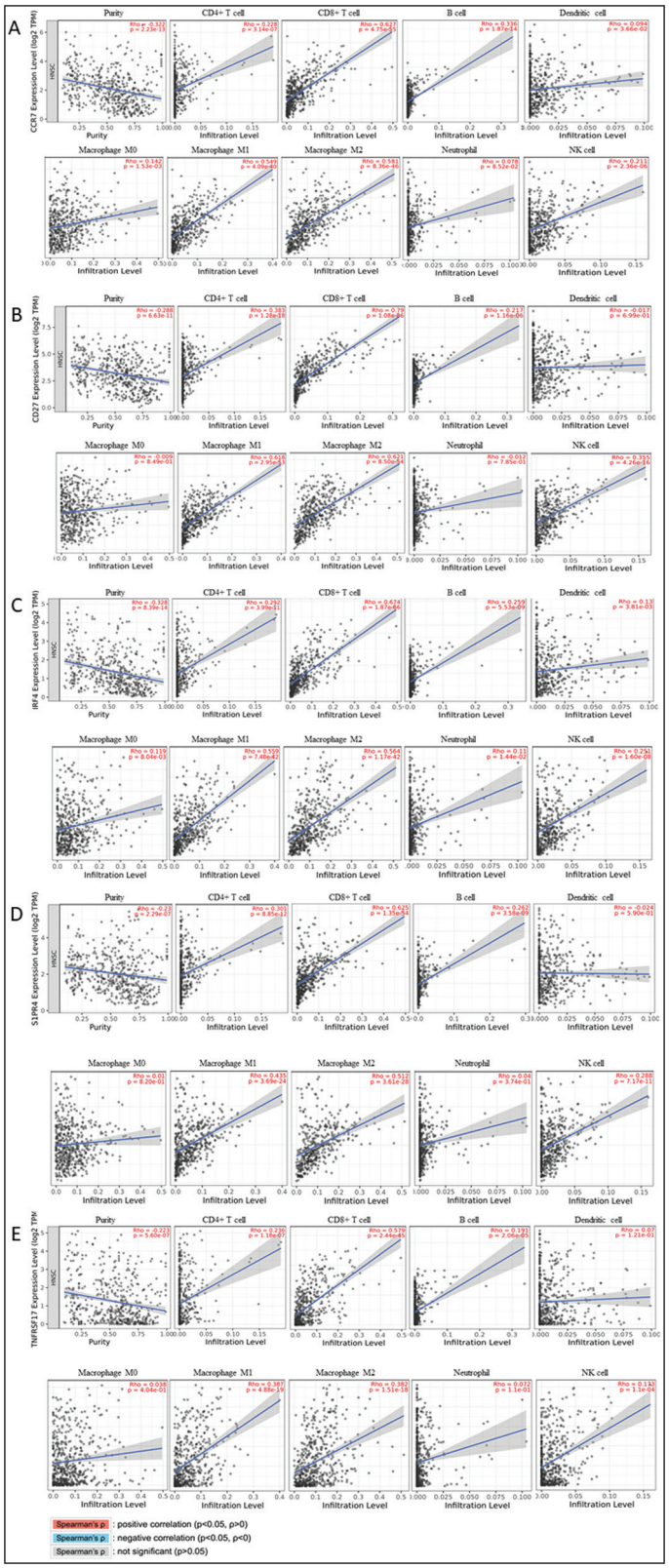


Figure 6: Correlation between hub-gene expression and immune cell infiltration determined using TIMER database. A value of $P < 0.05$ was considered statistically significant. The spearman's $\rho < 0$ and $\rho > 0$ were considered to indicate negative and positive correlations, respectively. (A) CCR7 expression showed a positive correlation with infiltration by all immune cells except neutrophils. (B) CD27 expression showed a negative correlation with infiltration by dendritic cells, M0 macrophages, and neutrophils but a presented positive correlation with $CD^{+}4$ T cell, $CD^{+}8$ T cell, B cell, M1 macrophage, M2 macrophage, and NK cell infiltration. (C) IRF4 showed a positive correlation with all immune cell types. (D and E) S1PR4 and TNFRSF17 expression showed positive correlations with all immune cell types except for dendritic cells, M0 macrophages, and neutrophils

rapidly increasing. OSCC has a high mortality rate and is difficult to detect in its early stages due to a lacking of effective early screening and monitoring methods.[17] OSCC is a tumor that is highly immunogenic and shows immune cell infiltration.[18] The formation and evolution of OSCC tumors may be influenced by a number of inflammatory mediators, including nuclear factor kappa-beta (NF-B), activator protein 1 (AP-1), tumor necrosis factor-alpha (TNF-), interleukin-6, -8 (IL-6, IL-8), and cyclooxygenase (COX)-2.[19] Tumor development, tumor growth, and metastasis are induced by inflammatory mediators.[20] Immune cell infiltration into the tumor causes persistent inflammation, which causes a gradual rise in inflammatory mediators during the OSCC tumor transformation process.[21]

Our PPI network analysis identified five hub genes that have high central betweenness and might play a role in OSCC. CCR7 has been associated with the ability of neoplastic cells to promote lymph node metastatic cancer and also promote the proliferation of neoplastic cells, as well as adhesion, migration, invasion, and angiogenesis in oral tumorigenesis.[22] IRF4 is a transcription factor of the IRF factor family that provides regulatory functions in the immune system and during oncogenesis. Previous reports have shown that IRF4 overexpression usually ameliorates tumor growth of colorectal cancer by promoting the transdifferentiation of T-regs into macrophage-like cells through the inhibition of BCL6 expression but acts as a tumor promoter in non-small-cell lung cancer (NSCLC).[23] TNFRSF17, CD27, and S1PR4 have been reported to be associated with tumor immunity, immune trafficking, and immune cell infiltration in tumors and may play a pivotal role in the immune response and inflammation in cancer. Although their exact role remains unclear, all five hub genes were discovered to be favorable prognostic markers in HNSCC.[24-26] The roles of these gene expression alterations in regulatory signaling during HNSCC development should be further studied in depth.

The pathophysiology of tumors is largely influenced by the immune system. Immune cells have a varied distribution throughout tumors and are connected to clinicopathological characteristics.[27] Microenvironments, such as the immune system invading nearby tumors, have an impact on treatment response and tumor growth.[28] tumor-infiltrating lymphocytes, which are regarded as the most important effectors of the host anti-tumor immune response that have been associated with increased survival in a number of cancer types. For HNSCC, an increase in regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) as well as a decrease in the number of T cells has been reported in addition to CD8+ effector T-cell dysfunction.[29] In a recent study, researchers investigated the gene expression patterns of tumor-infiltrating lymphocytes, including macrophages, neutrophils T cells, and B cells in various types of solid tumors.[30] Severe immune disruption was found to be the cause of impairment of the antitumor response in HNSCC. A strong correlation between the density and location of tumor-infiltrating lymphocytes may lead to better prognostic markers in cancer and could be used as a supplement to pathological characteristics of HNSCC.[31] Neutrophils and their precursors could be attracted by developing tumor cells that eliminate various factors such as CCL4, or CCL5 and IL-8.[32] Tumor-associated neutrophils were found to promote tumor growth by cancer metastasis, angiogenesis, immune suppression, and supporting genetic instability in OSCC.[33] NK cells have a di cytolytic effect through the production of granzymes, perforins, and interferon-γ (IFN-γ) that promote the activation of T-helper-1 (Th1) cells and myeloid cells.[34] $CD8^+$ lymphocytes are major effectors of the adaptive immune response that are involved in antitumor activity and tumor cell clearance.[35] An abundance of $CD8^+$ lymphocytes infiltrating tumors is not only a good prognostic marker in various cancers but also an argument for responsiveness in immunotherapy.[36] A previous study reported that tumors involving lip mucosa showed intense $CD8^+$ lymphocyte infiltration in tumor tissue compartments but there was no correlation with $CD4^+$ lymphocyte infiltration in OSCC.[37] However, both $CD8^+$ and $CD4^+$ activated lymphocytes express the programmed cell death protein-1 (PD-1) that exerts inhibitory effects when binding to specific ligands present on immune and tumor cells.[38]

For evaluation of our study, although the study employed a relatively large sample size, future research will be confirmed to validate these findings using samples from patients or patients' databases. Second, to better understand the functions of these hub genes would be the large-scale elucidation of the molecular processes of oral cancer.

Conclusion

In conclusion, our study used thorough bioinformatics analysis to search for DEGs in normal and OSCC tumor tissues. The study showed that inflammation and immunological response are related to OSCC development, indicating a change in biological function. Five hub genes, IRF4, CCR7, TNFRSF17, CD27, and S1PR4, were discovered by PPI network analysis. These genes show a positive link with CD4+ lymphocyte, CD8+ lymphocyte, B cell, and NK cell infiltration of

OSCC cancers. These genes' patterns of expression may serve as prognostic indicators in OSCC.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Authors contributions

JS and NK designed and provided a conceptual framework for this study. SC performed the bioinformatics analysis and contributed to writing the first draft of the manuscript. JS and NK made a substantial contribution to data collection and analysis. All authors revised and approved the final version of the manuscript.

Ethical policy and institutional review board statement

Not applicable.

Patient declaration of consent

Not applicable.

Data availability statement

The data during the analysis of this study are available from the corresponding author (Dr. Jutamaad Satayavivad, e-mail: jutamaad@cri.or.th) upon reasonable request.

References

1. Chen SH, Hsiao SY, Chang KY, Chang JY. New insights into oral squamous cell carcinoma: From clinical aspects to molecular tumorigenesis. Int J Mol Sci 2021;22:2252.
2. Caruntu A, Caruntu C. Recent advances in oral squamous cell carcinoma. J Clin Med 2022;11:6406.
3. Metgud R, Astekar M, Verma M, Sharma A. Role of viruses in oral squamous cell carcinoma. Oncol Rev 2012;6:21.
4. Vigneswaran N, Williams M. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 2014;26:123-41.
5. Huang S, Sullivan BO. Oral cancer: Current role of radiotherapy and chemotherapy. Med Oral Patol Oral Cir Bucal 2013;18:e233-40.
6. Ravi K, Suresh G, Prakash B, Sabitha K, Dhara P. Prognostic indicators of oral squamous cell carcinoma. Ann Maxillofac Surg 2019;9:364-70.
7. Kim S, Lee J, Park Y. The application of next-generation sequencing to define factors related to oral cancer and discover novel biomarkers. Life 2020;10:228.
8. Guo H, Li C, Su X, Huang X. A five-mRNA expression signature to predict survival in oral squamous cell carcinoma by integrated bioinformatic analyses. Genet Test Mol Biomark 2021;25:517-27.
9. Milano M, Agapito G, Cannataro M. Challenges and limitations of biological network analysis. BioTech 2022;11:24.
10. Dash S, Shakyawar S, Sharma M, Kaushik S. Big data in healthcare: Management, analysis and future prospects. J Big Data 2019;6:54.
11. Bao R, Huang L, Andrade J, Tan W, Kibbe W, Jiang H, *et al*. Review of current methods, applications, and data management for the bioinformatics analysis of whole exome sequencing. Cancer Inform 2014;13:67-82.
12. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, *et al*. DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res 2022;50:W216-W221.
13. Zhou G, Soufan O, Ewald J, Hancock R, Basu N, Xia J. NetworkAnalyst 3.0: A visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res 2019;47:W234-41.
14. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: An enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019;47:W556-60.
15. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, *et al*. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020;48:W509-14.
16. Liu Y, Pan B, Qu W, Cao Y, Li J, Zhao H. Systematic analysis of the expression and prognosis relevance of FBXO family reveals the significance of FBXO1 in human breast cancer. Cancer Cell Int 2021;21:130.
17. Coletta R, Yeudall W, Salo T. Grand challenges in oral cancers. Front Oral Health 2020;1:3.
18. Hadler-Olsen E, Wirsing A. Tissue-infiltrating immune cells as prognostic markers in oral squamous cell carcinoma: A systematic review and meta-analysis. Br J Cancer 2019;120:714-27.
19. Tampa M, Mitran M, Mitran C, Sarbu M, Matei C, Nicolae I, *et al*. Mediators of inflammation – A potential source of biomarkers in oral squamous cell carcinoma. J Immunol Res 2018;2018:1-12.
20. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, *et al*. Inflammation and tumor progression: Signaling pathways and targeted intervention. Signal Transduct Target Ther 2021;6:263.
21. Goertzen C, Mahdi H, Laliberte C, Meirson T, Eymael D, Gil-Henn H, *et al*. Oral inflammation promotes oral squamous cell carcinoma invasion. Oncotarget 2018;9:29047-63.
22. Rizeq B, Malki M. The role of CCL21/CCR7 chemokine axis in breast cancer progression. Cancers 2020;12:1036.
23. Wang J, Li S, Li H, Zhou X, Wen H, Lai B. IRF4 overexpression promotes the transdifferentiation of tregs into macrophage-like cells to inhibit the development of colon cancer. Cancer Cell Int 2021;21:58.
24. Bedognetti D, Hendrickx W, Marincola F, Miller L. Prognostic and predictive immune gene signatures in breast cancer. Curr Opin Oncol 2015;27:433-44.
25. Sarvaria A, Madrigal J, Saudemont A. B cell regulation in cancer and anti-tumor immunity. Cell Mol Immunol 2017;14:662-74.
26. Nema R, Kumar A. Sphingosine-1-phosphate catabolizing enzymes predict better prognosis in triple-negative breast cancer patients and correlates with tumor-infiltrating immune cells. Front Mol Biosci 2021;8:697922.

27. Fortis S, Sofopoulos M, Sotiriadou N, Haritos C, Vaxevanis C, Anastasopoulou E, *et al*. Differential intratumoral distributions of CD8 and CD163 immune cells as prognostic biomarkers in breast cancer. J Immuno Ther Cancer 2017;5:39.
28. Whiteside T. The tumor microenvironment and its role in promoting tumor growth. Oncogene 2008;27:5904-12.
29. Hendry S, Salgado R, Gevaert T, Russell P, John T, Thapa B, *et al*. Assessing tumor-infiltrating lymphocytes in solid tumors: A practical review for pathologists and proposal for a standardized method from the international immunooncology biomarkers working group: Part 1: Assessing the host immune response, TILs in invasive breast carcinoma and ductal carcinoma in situ, metastatic tumor deposits and areas for further research. Adv Anat Pathol 2017;24:235-51.
30. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema G. The immunosuppressive tumour network: Myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology 2013;138:105-15.
31. Paijens S, Vledder A, de Bruyn M, Nijman H. Tumor-infiltrating lymphocytes in the immunotherapy era. Cell Mol Immunol 2020;18:842-59.
32. Sionov R, Fridlender Z, Granot Z. The multifaceted roles neutrophils play in the tumor microenvironment. Cancer Microenviron 2014;8:125-58.
33. Masucci M, Minopoli M, Carriero M. Tumor associated neutrophils. Their role in tumorigenesis, metastasis, prognosis and therapy. Front Oncol 2019;9:1146.
34. Paul S, Lal G. The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy. Front Immunol 2017;8:1124.
35. Maimela N, Liu S, Zhang Y. Fates of CD8+ T cells in tumor microenvironment. Comput Struct Biotechnol J 2019;17:1-13.
36. Maibach F, Sadozai H, Seyed Jafari S, Hunger R, Schenk M. Tumor-infiltrating lymphocytes and their prognostic value in cutaneous melanoma. Front Immunol 2020;11:2105.
37. Caruntu A, Moraru L, Lupu M, Vasilescu F, Dumitrescu M, Cioplea M, *et al*. Prognostic potential of tumor-infiltrating immune cells in resectable oral squamous cell carcinoma. Cancers 2021;13:2268.
38. Waldman A, Fritz J, Lenardo M. A guide to cancer immunotherapy: From T cell basic science to clinical practice. Nat Rev Immunol 2020;20:651-68.