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Original Article

Oncology Section

ACE2 Centred Co-expression Network and Pathway Enrichment Analysis of Immune Regulatory Mechanisms in Oral Squamous Cell Carcinoma: An In-silico Study

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ABSTRACT

Introduction: Angiotensin-Converting Enzyme 2 (ACE2), the viral entry cellular receptor for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been recently known for its regulatory activity in Tumour Microenvironment (TME). ACE2 acts by local renin angiotensin dynamics through its influence on immune signalling and tissue remodelling. Even though the ACE2 expression is detected in Oral Squamous Cell Carcinoma (OSCC), its molecular role in OSCC remains unclear.

Aim: To identify the ACE2 associated immune networks and biological pathways in OSCC using transcriptomic analysis.

Materials and Methods: This was an in-silico transcriptomic and bioinformatic based observational study, was conducted in October 2025 in the Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. Transcriptomic data from the GSE9844 dataset (26 OSCC and 12 normal tongue samples) were analysed with an integrated bioinformatics approach. Gene-wise Spearman correlations with ACE2 were computed using R software (version 4.2.0), followed by preranked Gene Set Enrichment Analysis (FGSEA) and single-sample GSEA (ssGSEA) using the GSVA package (version 1.46.0) to identify Hallmark pathways enriched in ACE2 associated expression profiles. Leading-edge genes from overlapping pathways were used to construct a Protein–Protein Interaction (PPI) network in Search Tool for the Retrieval of Interacting Genes/Protein (STRING) database (version 11), and hub genes were determined by degree centrality.

Results: ACE2 expressions have shown statistically significant positive associations with immune-related pathways, including Interferon-α/γ (IFN), Tumour Necrosis Factor (TNF-α)/ Nuclear Factor kappa-light-chain-enhancer of activated B cell (NF-κB) signalling, Interleukin-6 (IL-6)-Janus Kinases (JAK)- Signal Transducer and Activator of Transcription 3 (STAT3) activation, and inflammatory pathways with a Spearman correlation coefficient (ρ) >0.25 and a False Discovery Rate (FDR) <0.05 in both enrichment algorithms. Molecular network analysis identified STAT 3, Vascular Endothelial Growth Factor A (VEGFA), Mitogen-Activated Protein Kinase (MAPK1) and Matrix Metalloproteinase (MMP) as key hubs regulating immune, angiogenic, and extracellular matrix remodelling processes in oral cancer. The ACE2 subnetwork has been identified adjacent to Dipeptidyl peptidase 4 (DPP4), Basigin (BSG) (CD147), and cytokines (IL-6, TNF), indicating potential crosstalk between ACE2 and inflammatory mediators. These findings show ACE2 within an IFN-driven, STAT3 centred regulatory framework in OSCC.

Conclusion: Present study highlighted that ACE2 is closely linked with IFN, inflammatory signalling pathways in OSCC, suggesting its role in regulating the tumour immune microenvironment. ACE2 gene is strongly associated with STAT3, VEGFA, MAPK1, and MMP9 that indicates the involvement in immune modulation and tissue remodelling, positioning ACE2 as a potential biomarker for immune activity and tumour progression in oral cancer.

Keywords: Angiotensin converting enzyme 2, Computational biology, Differential gene expression, Pathway analysis

INTRODUCTION

The ACE2 acts as a counter regulator to Renin-Angiotensin System (RAS) [1]. Together with its traditional role in controlling pulmonary and cardiovascular homeostasis, ACE2 regulates several biological processes including oxidative stress, inflammation, tissue remodelling, infiltrating tumours, IFN signalling, and the epithelial-mesenchymal transition, all of which are linked to the TME. OSCC is the most prevalent malignancy of the oral cavity and represents a significant clinical and public health challenge globally. According to Global Cancer Observatory (GLOBCAN) 2022, oral cancer ranks 16th in incidence and 15th in mortality worldwide [2]. Regardless of technological advancements in diagnostic and therapeutic approaches, OSCC is often diagnosed only at advanced stages, with high recurrence rate and poor overall survival due to limited resources which leads to 5-year survival rate nearly below 50% in many settings [3]. Its aggressive behaviour is mainly due to complex molecular alterations, that includes dysregulation of immune signalling, angiogenesis, extracellular matrix remodelling, and epithelial-mesenchymal transition [4,5].

Understanding the complexity of tumour molecular mechanisms underlying OSCC initiation and progression is crucial for the identification of prognostic biomarkers and the development of targeted therapeutic strategies. Recent advances in transcriptomics, single-cell profiling, and network biology now enable a deeper interrogation of tumour-intrinsic and stromal co-regulatory modules but significant knowledge gaps remain in translating these signatures to clinically actionable targets in OSCC. Several studies prove that ACE2 expression is implicated in tumour aetiology and act as a prognostic marker [6], despite conflicting results for diverse forms of cancer. In head and neck tissues, ACE2 is expressed in epithelial cells particularly in the olfactory mucosa, nasopharynx, salivary gland and in oral mucosa ACE2 is expressed in tongue, followed by buccal mucosa, gingival mucosa and to an extent in fibroblasts and immune cells [7]. Recent study has analysed that ACE2 was

significantly lower in OSCC tissues when compared with healthy samples, implying its potential tumour-suppressive roles [8].

Few recent studies demonstrate that ACE2 interacts with a broad network of molecular pathways. These interactions suggest that ACE2 could act as a hub integrating immune response, tissue remodelling, and vascular dynamics within the OSCC microenvironment [9]. The comprehensive mapping of ACE2 associated transcriptional and functional networks in oral cancer remains limited, and its precise mechanistic role inits molecular framework is not fully understood.

Importantly, despite current advances, three major gaps in understanding ACE2's molecular role in OSCC persist: a) The co-expression mechanism of ACE2 has not been systematically explored to identify genes that are regulated and the functional modules like immune, stromal, or metabolic modules accompanies the ACE2 expression pattern; b) The functional integration of ACE2 within the OSCC transcriptome landscape remains poorly defined. Analytical tools such as GSEA, ssGSEA, and Protein-Protein Interaction Networks (PPI) network mapping have rarely been utilised to find out its role in inflammatory signalling, IFN-mediated responses, or metastatic progression; c) The translational significance of ACE2-associated molecular networks in OSCC, including their relationship with tumour grade, immune cell infiltration, and clinical outcomes, has not been empirically established. Analysing these gaps is very crucial to know how ACE2 participates in tumour progression and its potential as a molecular biomarker or as therapeutic target in oral cancer.

Accordingly the study, aimed to determine the integrated molecular pathways associated with ACE2 in OSCC using bioinformatics and system biology techniques. Through transcriptomic profiling and interaction network framework, regulatory modules influenced by ACE2 that contribute to immune modulation, angiogenesis, and extracellular matrix remodeling can be analysed. The study systematically investigates OSCC transcriptomes using co-expression analysis, enrichment mapping, Spearman correlation ranking, GSEA, ssGSEA, PPI networks, and hub gene identification. Consequently, these multifocus analyses reveal key functional clusters, candidate genes, and signaling pathways underlying ACE2's role in oral tumour oncology.

MATERIALS AND METHODS

This was an in-silico, transcriptomic and bioinformatic based observational study, conducted in October 2025 in the Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India. Transcriptomic datasets were preprocessed and subjected to correlation analysis between ACE2 and genome-wide expression profiles. Genes showing significant association with ACE2 were analysed through both FGSEA and ssGSEA. Comparative evaluation of Normalised Enrichment Scores (NES) from FGSEA and ssGSEA determined ACE2 associated functional modules. Key network hubs and focus subgraphs were identified after leading-edge genes from important modules and they were extracted for network development. And hence the results underlined the predominant molecular clusters in OSCC that are influenced by ACE2.

Data source and preprocessing: Normalised gene expression matrix for human tongue SCC (38 datasets, including 26 tumour and 12 paired normal tongue controls; Gene Expression Omnibus (GEO) GSE9844, platform GPL570) were obtained from the GEO database. Low-variance probes were removed to reduce noise and ensure the reliability of downstream analyses. Variance filtering was performed using the Interquartile Range (IQR) thresholding method. Probes with IQR< 0.5 were excluded. Duplicate probes were handled by selecting the probe with the highest mean expression across the samples. As, all the samples were from single GEO dataset, no batch effect correction was required. However to ensure consistency, hidden confounding data was assessed by Principal Component Analysis (PCA), and no major associated clustering was observed. Gene symbols were mapped to MSigDB Hallmark gene sets (Broad Institute), retaining only genes present in these curated pathways. ACE2 expression data were extracted for each sample and used as the primary target gene for correlation and enrichment analysis.

Gene-ACE2 correlation analysis: Spearman's rank correlation coefficient (ρ) was calculated to identify genes co-regulated with ACE2, between ACE2 expression and each gene across all the 38 samples using R software (version 4.2.0). We applied the Benjamini-Hochberg (BH) adjustment for multiple hypothesis testing for multiple hypothesis testing on all genes to obtain FDR values. All genes were then ranked by their Spearman ρ with ties broken by mean rank to create an ACE2-centered gene list suitable for enrichment analysis. This approach enabled us to identify transcriptional modules that are potentially regulated by ACE2 in tongue squamous cell carcinoma.

Preranked Gene Set Enrichment Analysis (FGSEA): FGSEA was performed using the FGSEA algorithm that analyses NES efficiently. The Spearman-ranked gene list that is ACE2-centered gene list was used as input, for evaluating the enrichment of gene sets. This was performed using the MSigDB Hallmark gene sets. The FGSEA algorithm (fast GSEA) determined the NES for each set, with 10,000 permutations of gene labels (score Type="std"). Gene sets with fewer than 15 or more than 500 genes were excluded to avoid extreme size biases. Pathways with FDR <0.25 were considered significantly enriched in the ACE2 positive ranked list.

Single-sample GSEA (ssGSEA): In parallel, ssGSEA was applied (single-sample GSEA) using the GSVA package (version 1.46.0) to quantify pathway activity and an enrichment score on a per-sample basis, generating a matrix of NES values for each hallmark. Default parameters min Size=15, max Size=500, tau=0.25, a matrix of per-sample NES scores was obtained. We computed Spearman's correlation between its ssGSEA NES and ACE2 expression for each pathway across samples, with BH correction across for multiple comparisons. Pathways with FDR <0.05 were considered to be significantly associated with ACE2 at the sample level.

This combined method of pre ranked GSEA and ssGSEA enabled us to possibly identify pathways that were consistently linked to ACE2 throughout the individual sample level as well as the cohort.

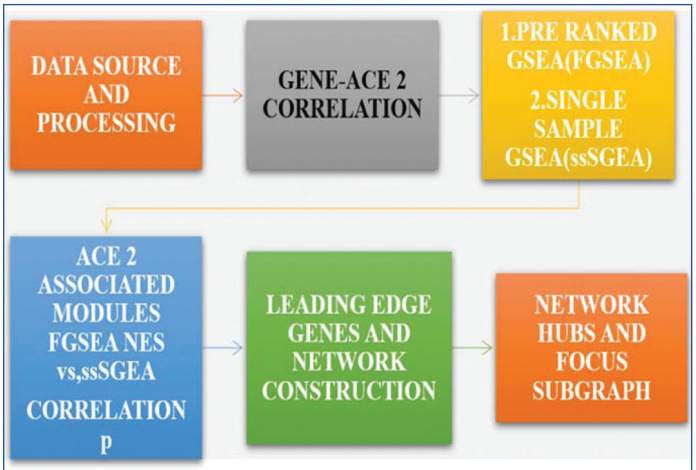
Identification of ACE2 associated functional modules: ACE2 associated functional modules were defined by intersecting significantly enhanced pathways from both pre-ranked GSEA and ssGSEA-ACE2 correlation analyses (both at FDR <0.25). This exacting approach ensured inclusion of pathways that are consistently enriched and correlated with ACE2 genes and those which are coherently associated with ACE2 expression across all the samples. Concordance between pre-ranked GSEA and ssGSEA ACE2 correlation analyses were visualised by plotting the NES from pre-ranked GSEA against the Spearman correlation coefficient (ρ) from ssGSEA, with pathway points coloured by $-\log_{10}$(FDR) from ssGSEA, that provided an integrated visualisation of enrichment strength, directionality, and statistical significance at the pathway level.

Leading-edge genes and network construction: For each common ACE2 associated pathway, we extracted the leading-edge genes representing the core contributors to maximum enrichment and they were combined. After removing duplicates, the leading-edge genes were pooled and mapped to their corresponding human Entrez protein IDs and submitted to the STRING database (version 11). This step was carried out to generate a high-confidence PPI network (with interaction confidence score ≥0.7; Species: Homo sapiens and no additional interactors were added to maintain specificity). Then the network topology was analysed to find out the degree (number of direct connections) and betweenness centrality (Importance of nodes in shorter paths) of each node using STRING's internal algorithms [10]. That resulted in identifying the hub proteins

that may regulate key ACE2 associated molecular interactions and functional connectivity in OSCC.

Network hub gene identification, Network visualisation and focus subgraph: From the complete interaction network, top 20 hub proteins were identified based on degree of centrality in the full network. A refined and focused subnetwork was generated comprising ACE2, its reported interactors namely STAT3, VEGFA, MAPK1, MMP9, along with their key IFN-associated nodes likes Interferon Regulatory Factor (IRF7), Interferon (alpha and beta) receptor 1 (IFNAR1), Interferon-Gamma Receptor (IFNGR) Protein Complex (IFNGR2), C-X-C motif chemokine ligand (CXCL10), their first neighbours, among the top 100 hubs. Then we constructed the subgraph with all their first neighbors in the network, and the top 100 hubs by degree and the subgraph was visualised using a spring (force-directed) layout, where node size represented degree, focus genes were highlighted in red, and edge thickness is proportional to STRING confidence values. To summarise the key network components, a bar plot depicting the top 20 hubs with emphasis on ACE2 related and IFN linked proteins was also constructed. Only top 20 hubs were selected to emphasise on most biologically relevant and strongly connected proteins. This approach increases practical interpretability and prevents false positive rating that leads to reduce network specificity.

This integrative and collective workflow systematically explored the ACE2 associated gene modules, enriched pathways, and interaction networks in OSCC [Table/Fig-1]. This combined transcriptomic and network analyses provided a mechanistic framework linking ACE2 to immune modulation and tumour progression that gives a foundation for understanding its functional significance in oral cancer biology.



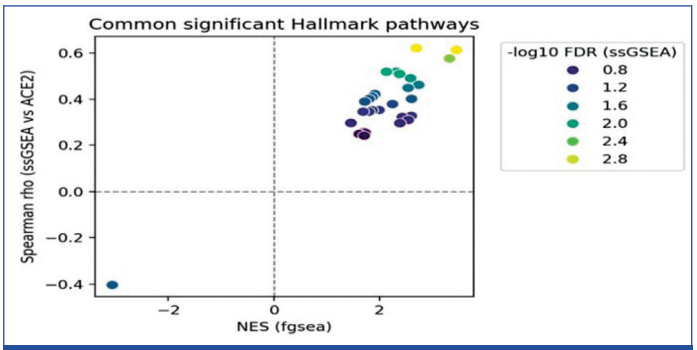
[Table/Fig-1]: Shows the workflow outlining ACE2-associated transcriptomic and network analyses in OSCC.

STATISTICAL ANALYSIS

The statistical analyses were performed using R software version 4.2.0. Correlation analysis was done using Spearman's Rank correlation test. Multiple testing correlations were applied using Benjamini-Hochberg FDR. Results were presented as NES values, Spearman ρ and FDR values.

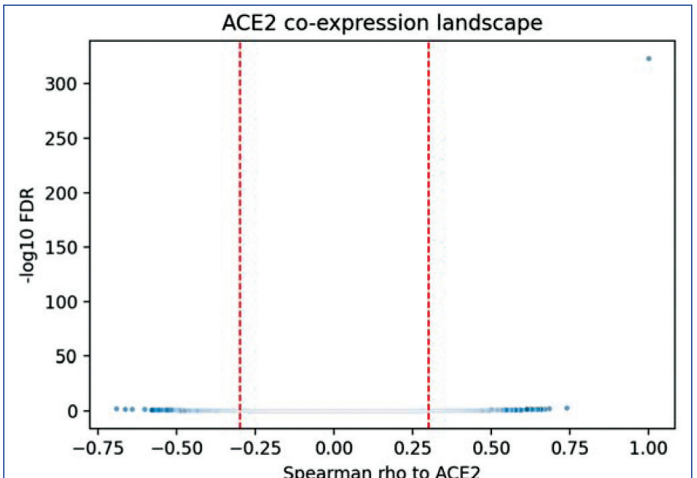
RESULTS

The ACE2 associated pathways are dominated by IFN and inflammatory signatures: An integrative enrichment analysis was performed to systematically identify biological pathways co-regulated with ACE2 in oral tissues, by combining FGSEA and ssGSEA. The intersection of these significant Hallmark pathways (FDR <0.05) from both approaches resulted a consensus set of ACE2-associated functional modules. Concordance between the pre-ranked GSEA NES and the ssGSEA correlation (Spearman ρ) highlighted the pathways that are both enriched and positively associated with ACE2 expression [Table/Fig-2]. Pathways located in the upper-right quadrant indicates high NES and high ρ primarily comprises IFN-related and inflammatory modules, including IFN-α response, IFN-γ response, inflammatory response, TNF-α signaling via NFκB, and IL6-JAK-STAT3 signaling. These included IFN-α response (NES ≈ 2.1-2.4), IFN-γ response (NES ≈ 2.3-2.6), inflammatory response (NES ≈ 2.0-2.2), TNF-α signaling via NF-κB (NES ≈ 1.9-2.1), and IL6-JAK-STAT3 signaling (NES ≈ 1.8-2.0). Analysis showed immune Hallmark pathways with positive enrichment (NES=+1.6 to +2.4) and moderate to strong correlation with ACE2 (ρ=0.38-0.62) all with significant adjusted p-values (FDR <0.05) [Table/Fig-2]. Stress response pathways, such as UV response and hypoxia, were also among the top hits, and in contrast most metabolic and cell cycle pathways exhibited weak or negative correlations with ACE2 [Table/Fig-2].



[Table/Fig-2]: Shows the consensus between pre-ranked GSEA NES and ssGSEA correlation coefficients for ACE2-associated Hallmark pathways. Each point in the Table/Fig represents a pathway. Positive NES and ρ values indicate the enrichment and positive correlation with ACE2. Immune pathways, especially Interferon (IFN) and inflammatory modules exhibit the strongest concordance.

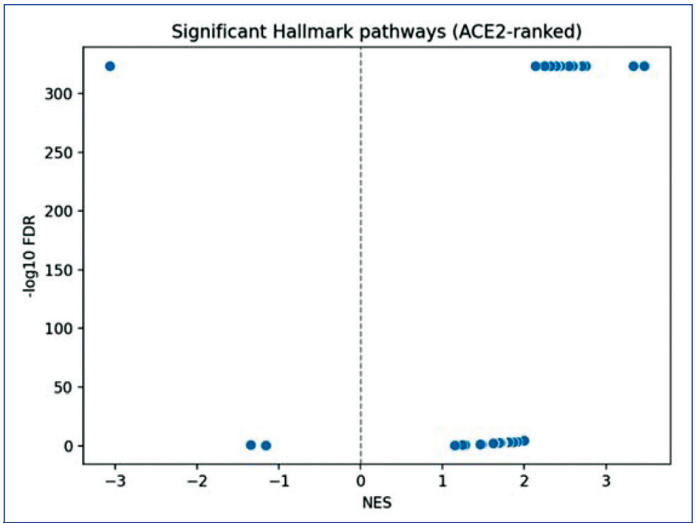
ACE2 co-expression landscape in oral tissues: Gene co-expression patterns relative to ACE2 were examined using the GSE9844 tongue SCC dataset. Gene co-expression profiling in the GSE9844 tongue SCC dataset exhibited specific transcriptional associations of ACE2. [Table/Fig-3] illustrates the relationship between Spearman correlation (ρ) with ACE2 and statistical significance (-$\log_{10}$ FDR). A Spearman correlation threshold of |ρ| ≥0.25 was applied as an analytical cut-off to define co-expression for subsequent network analysis. This cut-off represents a moderate correlation strength, which is commonly employed in transcriptomic co-expression analyses to balance sensitivity and specificity while reducing spurious associations in high-dimensional gene expression data. ACE2-associated gene transcripts with Spearman correlation coefficients ranging from ρ=+0.25 to +0.58 and high statistical confidence (–$\log_{10}$(FDR)=2.0-6.0; FDR <0.01). Negatively correlated genes were fewer and showed weaker effect sizes (ρ≥ –0.30). Genes that are positively correlated with ACE2 (ρ> +0.25) were predominantly associated with IFN signaling immune activation, and



[Table/Fig-3]: Gene expression correlated with ACE2 expression in tongue squamous cell carcinoma (GSE9844). The scatter plot illustrates Spearman correlation coefficients (ρ) versus statistical significance {-log10(FDR)}. Red dashed lines represent correlation thresholds of ±0.25, marking moderately correlated genes.

inflammatory response pathways indicating an immunoregulatory axis, whereas negatively correlated genes (ρ<–0.25) were aligned with metabolic and structural pathways, advocating a reciprocal regulation between immune and metabolic programs. These findings indicate that ACE2 expression in oral tissues are integrated with immune-inflammatory networks and are inversely linked to metabolic pathways. The presented ACE2 co-expression patterns correlate with the previous functional modules determined through enrichment and PPI network analyses, confirming ACE2's integrative role in tumour-associated signaling mechanisms.

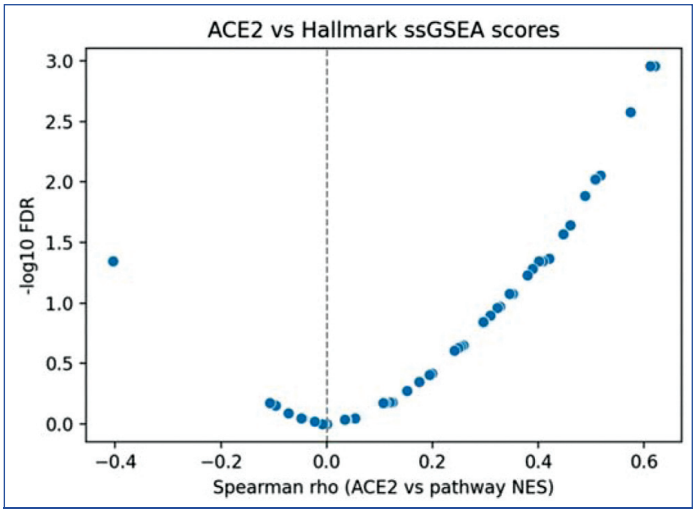
ACE2 immune associations through hallmark pathway enrichment: In order to understand the biological pathways associated with ACE2 expression, pre-ranked GSEA was performed using the ACE2-correlated gene set [Table/Fig-4]. Pre-ranked GSEA showed significant enrichment of immune and IFN pathways among ACE2-correlated genes (NES=+1.7 to +2.6; FDR <0.01). Metabolic pathways were significantly depleted (NES=–1.2 to –2.0; FDR <0.05). Pathways that exhibited positive NESs (with NES values ranging approximately from 1.8 to 2.6 and signaling, and IL6-JAK-STAT3 signaling. These pathways were significantly enriched among genes positively correlated with ACE2.



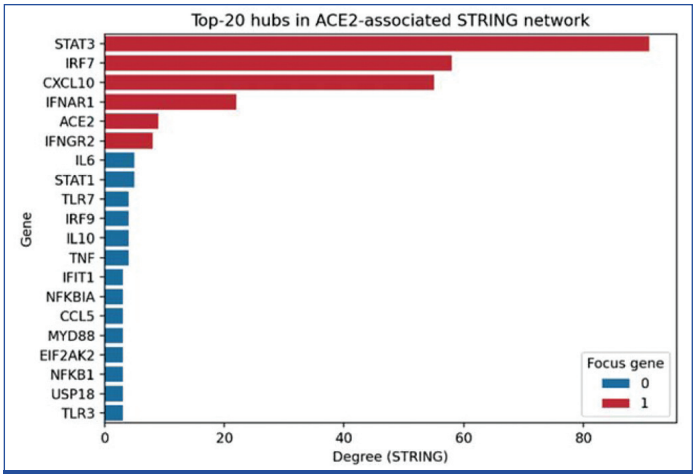
[Table/Fig-4]: Pre-ranked GSEA of ACE2-correlated genes. The x-axis represents the Normalised Enrichment Score (NES) and the y-axis denotes $-\log_{10}$(FDR). Positively enriched pathways highlight Interferon (IFN) and inflammatory signaling, whereas negatively enriched pathways indicate metabolic processes.

Single-sample GSEA confirms coordinated ACE2 immune activity: ssGSEA was performed at the individual sample level to validate pathway co-activation and to correlate ACE2 expression with pathway activity scores [Table/Fig-5]. ssGSEA pathway activity scores revealed strong positive associations with ACE2 expression (ρ=0.42-0.67) for IFN and inflammatory modules. These pathways demonstrated high statistical significance ($-\log_{10}$(FDR)=2.5-5.5; FDR <0.01). Pathways that are positively correlated with ACE2 with ρ-value>0.25 include IFN-α/γ response pathway, IL6-JAK-STAT3 pathway, TNF-α/NF-κB signaling pathway, and general inflammatory response pathways. These finding supports the GSEA and network-based findings by demonstrating the consistent ACE2 expression link to innate immune activation and inflammatory signaling across oral tissue samples.

Construction of an ACE2 centered Protein-Protein interaction (PPI) network: PPI network hubs were identified based on degree centrality, reflecting the number of direct interactions per protein. High-confidence interactions were retained, and network topology was further analysed to identify the central hub proteins that may coordinate key signaling modules. The top 20 hub proteins were determined by degree according to network degree centrality [Table/Fig-6]. Among the all protein hubs, STAT3 has been demonstrated the most interconnected hub, linking IFN/inflammatory pathways like cytokine signaling and other growth factor pathways. ACE2,



[Table/Fig-5]: Correlation of ACE2 expression with Hallmark pathway activities using single-sample GSEA (ssGSEA). Each point represents a pathway, with the x-axis showing the Spearman correlation (ρ) between ACE2 expression and pathway activity scores (NES), and the y-axis representing $-\log_{10}$(FDR). Table/Fig depicts a strong positive association of ACE2 with Interferon (IFN) and inflammatory modules.

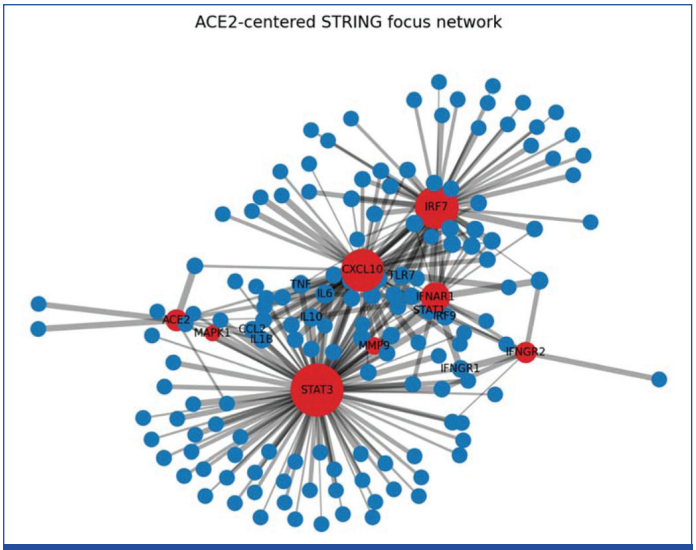


[Table/Fig-6]: Illustrates the ACE2-centered PPI network and top 20 hubs ranked by degree. Node size reflects the number of interactions. Nodes correspond to ACE2, STAT3, VEGFA, MAPK1, MMP9, and Interferon (IFN)-related proteins are highlighted. The STRING network structure highlights STAT3's significance as a connecting hub between immune and growth-factors, while ACE2 placement next to important receptors and cytokines underlines its relation to Interferon (IFN) and inflammatory signalling.

VEGFA, MAPK1, and MMP9 were ranked as primary hubs, together with the other additional key gene hubs. ACE2 was positioned near significant membrane receptors and cytokine nodes such as DPP4, BSG, IL6, and TNF. ACE2 exhibited moderate connectivity, which suggests its important role in immune-stress signalling. IRF7, IFNAR1, IFNGR2, and CXCL10 that acted as supplementary hubs were examples of IFN-associated proteins, highlighting the significance of the IFN network. VEGFA linked to angiogenesis-related interactions, MAPK1 is anchored to MAPK signaling, and MMP9 connected extracellular matrix remodeling to inflammatory pathways.

This network thus highlights that ACE2 participates in an integrated system connecting immune activation, cytokine signaling, tissue remodeling, and growth factor pathways.

Identification of key network hubs connecting ACE2 to immune and growth-factor pathways: ACE2 centered STRING network [Table/Fig-7] revealed STAT3 as a central hub with high betweenness centrality, bridging IFN/inflammation and growth factor signaling clusters. While, ACE2 is moderately connected, but its connectivity is surrounded by high-confidence interactors including membrane receptors like DPP4 and BSG, as well as cytokines IL6 and TNF. Although ACE2 does not exhibit the highest degree, its moderate connectivity places it in close proximity to key immune receptors, cytokines, and IFN-related proteins, indicating



[Table/Fig-7]: Visualises the ACE2-centered PPI network, with node size proportional to degree and edge thickness representing STRING confidence. Focus genes (red) include ACE2, STAT3, VEGFA, MAPK1, MMP9, and key Interferon (IFN) nodes, that emphasises their centrality.

a biologically relevant integrative role rather than a dominant signaling hub. ACE2 is positioned within a functional network with neighborhood that consisted of immune receptors, cytokines, and IFN-associated proteins, ACE2 as an interface linking immune, inflammatory, and stress-response signaling pathways rather than implying independent network control. IFN-associated nodes IRF7, IFNAR1, IFNGR2, and CXCL10 clusters were proximal to ACE2, emphasising the IFN-driven module. VEGFA and MAPK1 occupy angiogenesis and MAPK signaling submodules, respectively, while MMP9 connects extracellular matrix remodeling to inflammatory pathways. These findings indicate that ACE2 functions as a multifaceted component of an immune activation, angiogenesis and matrix remodeling nodal nexus in oral tissue biology.

DISCUSSION

ACE2 plays an array of functions along with its primary role in cardiovascular and pulmonary regulation. ACE2 counterbalances the oncogenic ACE/Ang II/AT1R axis by generating Angiotensin (1-7) {Ang(1–7)}, which exerts anti-inflammatory, antifibrotic, and antiproliferative effects [11]. Several studies have demonstrated that ACE2 expression is altered in multiple epithelial malignancies, particularly those arising from mucosal tissues, including the respiratory, gastrointestinal, and oropharyngeal tracts [12,13]. Further experimental evidence also show that downregulation of ACE2 enhances tumour progression and metastatic behaviour, emphasising the protective functions of the ACE2/Ang-(1–7)/Mas axis [14].

However, the variations and dysregulation in ACE2 expression reported across previous cancer studies may be due to several biological factors: a) Patient specific variations in tumour site, grade, differentiation, epithelial integrity and cellular phenotype shift across the lesions [9]; b) The immune microenvironment plays a crucial role [11,12]; c) In addition, as tumours progresses ACE2 level might change from early dysplasia to advanced carcinoma [13-15]; d) External factors including microbial load, chronic inflammation might also modify ACE2 regulatory mechanisms [9,11-15]. Collectively, these factors underline the heterogeneity in ACE2 expression observed across cancer cohorts.

An earlier study by using Enzyme Linked Immuno Sorbent Assay (ELISA) approach demonstrated that ACE2 is significantly low in OSCC tissues when compared to normal oral mucosa [8]. Contrastingly, Reverse Transcription-Polymerase Chain Reaction (RT-PCR) analyses reported increased ACE2 mRNA expression in OSCC tumour samples [15]. This disparity between transcript and protein levels depicts a complex regulatory landscape. It could be due to post-transcriptional mechanisms that includes altered mRNA stability. Specifically, Head and Neck Squamous Cell Carcinoma (HNSC) have depicted a distinct molecular subgroup with unique ACE2 expression patterns [16].

Hence, the current study provides a combinative analysis of ACE2 associated transcriptional programs in oral tissues, demonstrating that ACE2 expression in the oral mucosa and OSCC is tightly linked within IFN-driven and inflammation-linked signaling networks [Table/Fig-2]. These observations show that ACE2 expression in oral tissues is closely related to innate immune and inflammatory signaling particularly IFN-driven signaling. These results are consistent with previous reports of ACE2 as an IFN-stimulated gene in epithelial cells. Mapping statistical significance using $-\log_{10}$ (FDR) colour indicates that the most statistically significant pathways are immune-related. Together, these analyses reveal a coherent ACE2-centered functional module dominated by IFN and inflammatory signals.

Genes that showed a positive correlation with ACE2 ($\rho>0.25$) were largely enriched for IFN signaling, immune activation, and inflammatory response pathways, suggesting that ACE2 expression is actively associated with immunoregulatory axis [Table/Fig-3]. By combining gene co-expression profiling, pathway enrichment, and network topology analysis approaches, the present study showed that ACE2 not only acts as an epithelial receptor but also participates in the wide range of immune-inflammatory pathways that involves in the TME [Table/Fig-4]. On the other hand, negatively NES enriched pathways were mainly downregulated by metabolic and biosynthetic processes indicating downregulation of metabolic programs in ACE2 high conditions. These findings support the association of ACE2 with immune activation, IFN signaling, and inflammatory stress responses in oral tumour tissues, and may act as an immunomodulatory node role in the TME.

A key finding of the present study was that ACE2 is co-expressed along with the pathways related to IFN signaling and inflammation. Both type I (IFN-α) and type II (IFN-γ) immune responses were significantly increased, together with IL-6/JAK/STAT3 and TNF–NF-κB factors [Table/Fig-5]. These results were in line with the well-known concept that the ACE2 is an Interferon-Stimulated Gene (ISG), particularly in mucosal epithelia [17,18].

Evidences shows that STAT3 is strongly associated in the progression of OSCC and has a role in therapeutic resistance, including the reduced responsiveness to chemotherapy, radiotherapy, and Epidermal Growth Factor Receptor (EGFR)-targeted agents [19]. Aberrant activation of the STAT3 signalling axis promotes an immunosuppressive TME in OSCC [20]. This occurs through the enhanced secretion of key cytokines such as TGF-β1, VEGF, IL-6, and IL-10, which collectively impair cytotoxic T-cell activity and enable tumour cells to evade immune recognition and elimination [21-26].

Integrated analysis of leading-edge genes from significant Hallmark pathways using STRING based PPI network, revealed STAT3 as the most interconnected hub linking IFN-driven inflammation pathways, cytokine signaling pathways and growth factor pathways. Along with STAT3, ACE2, VEGFA, MAPK1, and MMP9 were also ranked as major hubs, emphasising their vital roles in interacting immune, angiogenic and stress response pathways [Table/Fig-6,7].

In the current study, enrichment of IFN signatures associated with ACE2 is validated by both pre-ranked GSEA and ssGSEA. The results suggest that ACE2 induction in oral tissues may occur as part of a coordinated antimicrobial response. This was consistent with single cell studies in respiratory and oral epithelia showing that IFNs robustly upregulate ACE2 expression, particularly its “ISG-ACE2” truncated isoform [18,27].

This co-expression analysis is first of its kind, providing a novel integrating perspective of ACE2 in OSCC. The relative transcriptional context of ACE2 is strongly related to immune-inflammatory

pathways, irrespective of ACE2 expression. This study furthermore strengthens and validates the ACE2's central role in OSCC as a linkage axis between IFN signaling and inflammatory networks within the TME. Co-expression profiling in the study demonstrated strong correlations (ρ>0.25) with type I/II IFN responses, IL-6/JAK/STAT3, and TNF-NF-κB pathways, positioning STAT3 as a key hub along with ACE2, VEGFA and MMP9 that drives immune activation, angiogenesis, and remodeling. This suggests that tumours with decreased ACE2 expression may represent immuno suppressive and aggressive subtypes, whereas ACE2 high level tumours may signify more active immune TMEs. The enrichment of IFN and inflammatory modules implies that ACE2 may identify a subpopulation of OSCC with increased innate immune activation or chronic inflammation, both of which could influence treatment response and metastatic routes of progression.

The functional association of ACE2 with IFN responses, STAT3-centred networks suggest ACE2 may reflect the immune activation status of the TME. ACE2 interacts with key regulators of angiogenesis, extracellular matrix remodelling, including VEGFA and MMP9, indicating potential role in tumour progression and invasiveness. Clinically, these findings suggest ACE2 associated pathways could impact its responsiveness to immune modulatory and anti-inflammatory therapeutic strategies in OSCC

The findings of the present study provide foundational framework on ACE2 associated immune networks in OSCC. Studies focusing on ACE2's role in OSCC should include considering the mechanistic, its interaction with TME and clinical factors to understand its complete biological and therapeutic relevance. Cell line assessment of ACE2 in OSCC will help in identifying its role on IFN signaling, epithelial mesenchymal transition, cell migration, angiogenic activity, and cytokine regulation. Co-culture models may help in understanding how ACE2 influences the immune functions like macrophage and T-cell polarisation. Cohort studies are also required to determine the specificity and sensitivity of ACE2 as prognostic or diagnostic marker for immunotherapy response.

Limitation(s)

Despite its strengths, this study had few limitations: a) The study was based on bulk transcriptomic datasets with modest sample size, which lacks cellular resolution and precise mapping of ACE2 expressing cells; b) Co-expression and network correlation analysis alone cannot provide us with the causation that lacks experimental validation; c) Study was based on single public dataset that lacks independent validation. Hence, Single cell RNA-seq studies are required to determine whether ACE2 expression arises mainly from malignant epithelial cells, basal progenitors, or from infiltrating immune and stromal cell populations.

CONCLUSION(S)

The study analysed the transcriptomic data from the GSE9844 cohort containing 26 OSCC and 12 normal tongue samples using an integrated bioinformatics approach. Integration of Spearman correlations, pre-ranked GSEA, and ssGSEA approaches identified ACE2 associated expressions are enriched with IFN and inflammatory signaling pathways. The Leading-edge genes from these pathways were used to construct a PPI network, where the key hub genes include STAT3, VEGFA, MAPK1, and MMP9 implicating ACE2's role in immune modulation and tissue remodeling. Collectively, these findings suggest ACE2 as a key node that influences the tumour-immune interactions and microenvironmental mechanism in OSCC.

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