In Silico Analysis of Differentially Expressed Gene Sets Reveals LY6E as a Potential Candidate Gene in Oral Squamous Cell Carcinoma (OSCC) Progression.

Abstract

Oral Squamous Cell Carcinoma (OSCC) is mostprevalent head and neck cancerand potentially malignant in oral cavitycancer. OSCC is asymptomatic in the early stages and mostly diagnosed atlate-stage that significantly hinders successful treatment. Genetic mutations may also cause cancer development in oral cavity; however, no specific genes or markers has been identified for OSCCs progression. While a few studies have reported some molecular markers like Cyclin D1 and BCL2, the lack of specific early diagnostic and prognostic markers remains the biggest obstacle for OSCC therapy till date. This study has been designed to identify specific molecular markers for precise diagnosis and prognosis of OSCC for successful treatment. Understanding the complex molecular mechanisms involved in OSCC pathogenesis is crucial to address this challenge effectively. The study utilizes a comprehensive approach to examine the differentially expressed genes (DEGs) in a large OSCC dataset (GSE85195) obtained from the Gene Expression Omnibus (GEO) database. Upon analysis of datasets, we found 245 common DEGs in all four stages of cancer within the dataset.Further network analysis of 245 DEGs revealed that 10 genes are highly connected based on the highest MCODE (Molecular Complex Detection) score. Among all 10 genes, LY6E geneshowed highest MOCODE value, indicatingLY6E may becritically involve in OSCC progression and act aspromising candidate biomarker. To delineate the molecular mechanism of gene regulation of LY6E, we further looked into its interactions with microRNAs (miRNAs) and transcription factors (TFs). Our findings identified certain miRNAs and TFs that may regulate LY6E expression, indicates its involvement in OSCC development. This study emphasizes the potential role of LY6E in OSCC progression and suggests LY6E could serve as a valuable diagnostic tool. These findings lay the groundwork for future research to develop targeted therapies based on the LY6E signaling network, which could ultimately improve clinical outcomes for OSCC patients.

Keywords: Oral Squamous Cell Carcinoma (OSCC), Differentially Expressed Genes (DEGs), Extracellular Matrix (ECM) Receptor Interaction, Matrix Metalloproteinases (MMPs), Signaling Pathways (e.g., IL-17, Wnt, CCKR)

Introduction

Oral Squamous Cell Carcinoma (OSCC) represents the leading cancer type in the oral cavity, comprising more than 90% of the cases [1].It occurs due to the uncontrolled growth of altered squamous epithelial cells that line the oral cavity.It is the sixth most common cancer in the world [2, 3]. The majority of oral cancer cases are found in South Asia, primarily due to the prevalent habits of smoking, alcohol consumption, and betel quid chewing in the region [4].Oral cancer is 2 to 3 times more frequent in men than in women. In India, it ranks second in terms of incidence, after breast cancer, and third in terms of mortality, following breast and cervix uteri cancer.Cancers originating in the lip and oral cavity represent major contributors to cancer-related morbidity and mortality, accounting for an incidence rate of 10.3% and a mortality rate of 8.8%, according to Globocan 2020 data(https://gco.iarc.fr).The primary causative factors for the development of oral cancer are tobacco and alcohol consumption, estimated to be responsible for 90% of cases[3, 5].Additionally, the Human Papillomavirus (HPV) and Epstein-Barr virus (EBV) are also crucial biological risk factors for oral cancer development[6].

The development of OSCC is a multistep process modulated by endogenous as well as environmental factors and involves changes in normal mucosa accompanied by invasion and distant proliferation to lymph node [7][8]. During the development of oral cancer, multiple genetic and epigenetic events occur that alter the normal functions of oncogenes and tumor suppressor genes, resulting in the progression[9][7]. Interferon beta (IFN-β) is critical in the body's defense against diseases and impacts cancer progression and viral infections[10]. It regulates the immune system and inhibits viral replication, thereby reducing the risk of virus-associated carcinogenesis[11]. The LY6E gene, which is involved in immune response regulation, has been identified as a significant marker in Oral Squamous Cell Carcinoma (OSCC) research, suggesting a potential role in cancer progression through immune evasion mechanisms[12]. IFN-β's interaction with LY6E could be pivotal, especially since LY6E influences the tumor's ability to evade the immune system[13]. This relationship is further complicated by the role of viruses in cancer development, where LY6E's modulation of the immune response against viruses could intersect with cancer therapy, particularly in therapies aiming to bolster immune surveillance[14].

Detecting oral cancer in its early stages and potential treatments is considered the most efficient way to improve patient survival. The 5-year survival chance is less than 50% in late-stage diagnosis. As a general rule, the prognosis of disease worsens with the advancement [15][16]. However, if diagnosed in an early stage, the survival rates can exceed 80%[16]. Regardless of all favorable therapeutic advancements in the field of oral cancer, there are no targeted curative treatments,and overall survival remains at a disappointingly stable level[17].Several molecular markers implicated in the carcinogenesis of OSCC have been evaluated by many investigators, including molecules involved in cell cycle regulation, apoptosis, angiogenesis, DNA repair system, and degradation of extracellular matrix. However, this evidence remains inconclusive; to battle the poor morbidity and mortality rate associated with OSCC, there is a great need to evaluate more effective targeted treatment options[18][19]. Currently, the main conventional prognostic factors for the survival of oral squamous cell carcinoma patients are the histological identification of tumor at the time of diagnosis; however, recent studies use many prognostic biomarkers from the body fluids of oral cancer patients which are known to influence the oncological outcomes [20][21].

Nowadays, cancer research is focused on understanding the underlying mechanisms, which can be crucial to finding novel pharmacological markers for the treatment of OSCC. Yet not all aspects of the progression of tumor have been fully understood[22][23]. Moreover, identifying novel molecular markers for early detection, prognosis stratification, and therapeutic evaluation is a continuous challenge[24]. However, various studies on markers are available. Still, thenumerous molecular genes are tightly connected in migration, proliferation, apoptosis, and metastasis, which makes it challenging to understand the OSCC mechanism and detection at the early stage [23]. Identifying improved targeted treatment and prognostic markers are significant priorities in oral cancer. It requires knowledge oforal cancer's genetics and molecular biology [25].

Therefore, with this study, we hope to shed light on promising biomarkers and their role in OSCC progression that has not been explored yet.There is a significant clinical need to build a model for developing novel targeted approaches against oral cancer.This study analyzed the oral cancer dataset GSE85195, which had 34 OSCC, 15 pre-cancer, and 1 control sample. A total of 245 genes were identified, which is common DEGs. Further analysis revealed that 10 genes are highly connected based on the highest MCODE score, indicating that LY6E is a promising molecular driver for oral cancer. The study highlighting LY6E as a candidate biomarker for oral cancer underscores the need to understand how LY6E's regulation of immune responses could be leveraged in therapeutic contexts, possibly enhancing

immunotherapy's effectiveness. The connection between IFN-β, LY6E, and cancer progression underscores the potential for new therapeutic approaches by targeting these mechanisms, especially in cancers with viral involvement or where immune evasion is a principal concern. This approach may lead to the development of target-dependent tumor therapy.

Materials and Methods

Dataset Analysis

Gene expression profiles for 50 oral cavity samples, including 34 cases of oral squamous cell carcinoma (OSCC), 15 cases of oral leukoplakia (OLK), and 1 control sample were collected from the NCBI-GEO database, available at (http://www.ncbi.nlm.nih.gov/geo/). These profiles were gathered using the GSE accession ID GSE85195 and are based on the GPL6480 platform.

Identification of DEGs

The GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) tool was utilized to categorize samples into relevant groups (Control, Stage 1, Stage 2, Stage 3, and Stage 4) and to facilitate comparisons between these groups. The analysis of differential expression was conducted using the GEOquery and Limma tools. GEOquery serves to parse GEO data into R data structures, while limma (Linear Models for Microarray Analysis) acts as a statistical test designed to pinpoint differentially expressed genes in microarray data. The sorting of differentially expressed genes (DEGs) was based on the LogFC10 value (+/- >1) and the adjusted p-value. DEGs among the different groups (Control, Stage 1, Stage 2, Stage 3, and Stage 4) were identified, and those common across all groups, including the control, were compared to uncover common gene signatures.

Network analysis and module extraction

Identified gene sets were fed to the STRING (https://string-db.org/), and the protein-protein interactions (PPI) were visualized among them using Cytoscape v 3.8.2 (https://cytoscape.org/). Gene pairs with a combined score greater than 0.4 were considered for networking. The top modules were extracted using the MCODE (Molecular Complex Detection) plugin of Cytoscape. Parameters taken for the module extraction were degree cutoff= 2, Node score cutoff = 0.2, K-score = 2, and Max. depth=100.

Gene ontology and KEGG pathway enrichment analysis of DEGs

The significant enrichment analysis of DEGs was assessed in this study based on Gene ontology (GO) using gProfiler (https://biit.cs.ut.ee/gprofiler/gost), a biological database of gene/protein families and a free online tool for functional annotation analysis. GO analysisincludes the following categories: biological process (BP), cellular component (CC), molecular function (MF), and panther pathway (PP). The gProfiler is a common and useful method for annotating genes and gene products and identifying characteristic biological attributes of high-throughput genome or transcriptome data. KEGG pathway enrichment analysis was performed using the gProfiler platform in the biological context of differentially expressed genes.

Expression profile, stage plot,and survival analysis

The expression of identified biomarkers was further validated with TCGA and GTEx datasets of Liver hepatocellular carcinoma in 369 tumors and 160 normal cases using the GEPIA (http://gepia.cancer-pku.cn) tool. Log2FC cut-off and p-value cut-off were taken as 1 and 0.01, respectively. Tumor samples were indicated in red while normal samples were indicated in grey. Survival analysis was also performed using the GEPIA tool. Overall survival with a median group cut-off at a 95% confidence interval and a cut-off high as low as 50% was taken as criteria. Overall survival analysis for each gene selected based on the MCODE score was performed.

Transcription factor prediction analysis

Transcription factors and biomarkers-related micro-RNAs have been identified using the Network analyst tool (https://www.networkanalyst.ca/). Networkanalyst is used for gene/protein list. The gene list is pasted in the gene list input tab; *Homo sapiens* were selected and uploaded for analysis.

Results

Uncovering 245 key DEGs in oral squamous cell carcinoma (OSCC) progression.

The GSE Id GSE85195 expression profile is analyzed using GEO2R with the GEO-query and limma package, grouping samples into Control, Stage 1, Stage 2, Stage 3, and Stage 4. The box plot (Fig. 1A) demonstrates the normalization of sample data within the dataset, offering a clear view of distribution and central tendencies. The UMAP plot in (Fig. 1B) provides a detailed visualization of relationships between different sample groups, including those that

appear distantly connected. The volcano plot (Fig. 1C) and the MD-Plot (Fig. 1D) reveal substantial evidence of differential gene expression across all sample groups, highlighting significant genetic variations and patterns. Our analysis identified sets of common DEGs in the dataset GSE85195 based on both magnitude of change (log2 fold change +/-1) and statistical significance (adjusted p-value <0.05) in all comparisons: Control vs. Stage 1 (318 DEGs), Control vs. Stage 2 (10012 DEGs), Control vs. Stage 3 (9392 DEGs), Control vs. Stage 4 (4480 DEGs). Further comparison employing Venn diagrams revealed 245 significantly differentially expressed genes (DEGs), which will serve as early biomarkers of OSCC as these are present in the control, early stage as well and advanced stages of OSCC (Fig. 1E).

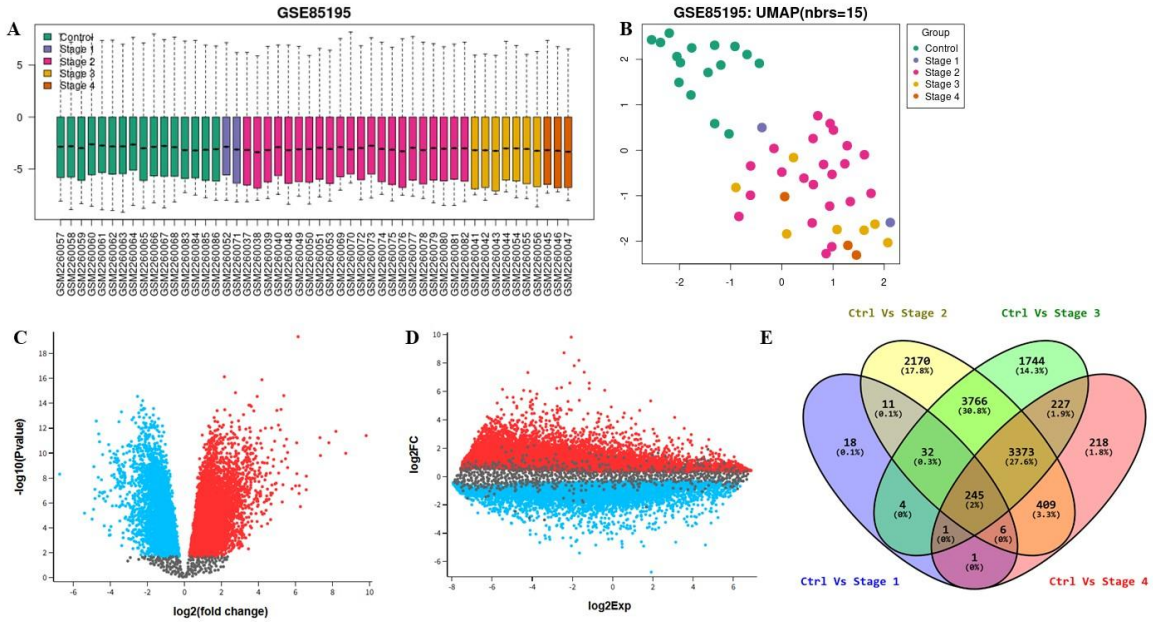

Figure. 1: Expression Analysis of DEGs: *A- Data Normalization Box Plot, B- UMAP for Group Expression, C- Volcano Plot, D- MD Plot, E- Venn Diagram For Common DEGs in all stages of Oral Cancer.*

Construction of network, analysis, and extraction of modules

All 245 DEGs were then subjected to STRING analysis (https://string-db.org/) and network analysis by Cytoscape. STRING analysis resulted in a protein-protein interaction network with a combined score>0.4. The network was visualized further in Cytoscape, and module extraction was performed using the MCODE plugin. Our data analysis revealed a highly interconnected module of 10 DEGs with an MCODE score of 10, suggesting a potential candidate for OSCC progression. These 10 DEGs include USP18 (Ubiquitin specific peptidase 18), OAS2 (2'-5'-oligoadenylate synthetase 2), LY6E (Lymphocyte antigen 6 family member E), ISG15 (Interferon-stimulated gene 15), IRF7 (Interferon regulatory factor 7), IFIT3

(Interferon Induced Protein With Tetratricopeptide Repeats 3), IFI6 (Interferon-alpha inducible protein 6), IFI35 (interferon-induced protein 35), HERC6 (HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 6), BST2 (Bone marrow stromal antigen 2) (Fig 2). Subsequently, the 10 genes within this module with the highest individual MCODE scores were selected for further pathway analysis.

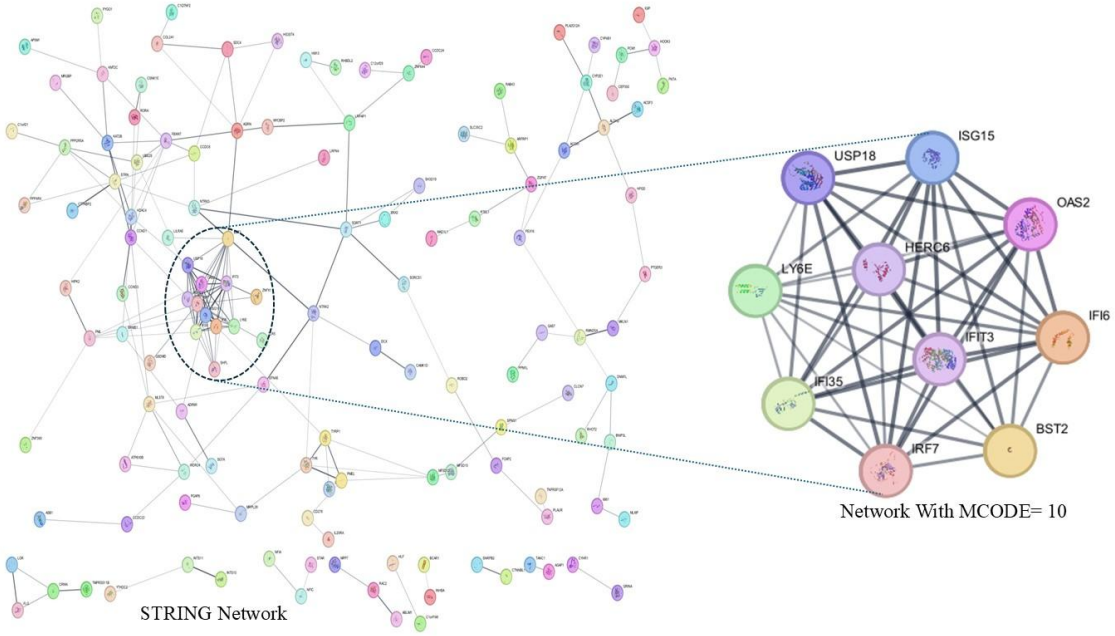

Figure. 2:Analysis and Extraction of Modules with Highest MCODE Score. *Network visualization from STRING analysis showing modules with the highest MCODE score of 10. Nodes are color-coded to highlight these high-score modules, emphasizing their dense interconnections and central role in the network.*

Gene ontology (GO)analysis of key DEGs in OSCC

Unveiling the functional underpinnings of OSCC progression, Gene Ontology analysis of these 10 high-scoring STRING modules (MCODE score 10) was performed. GO analysis performed by gProfiler tools revealed a remarkable enrichment for processes critical for cancer cell proliferation and cell motility (Fig. 3, Table 1). These genes, intimately linked to the biological process involved in interspecies interaction between organisms, negative regulation of the viral process, and positive regulation of interferon-beta production, seem to likely play a pivotal role in orchestrating the uncontrolled growth and invasive behavior of cancerous cells. This convergence of key regulatory functions in a single module highlights its potential as a promising therapeutic target for combating this aggressive disease.

ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:BP	GO:0044419	biological process involved in interspecies interact...	1.592×10^{-8}
2	GO:BP	GO:0048525	negative regulation of viral process	9.566×10^{-5}
3	GO:BP	GO:0032728	positive regulation of interferon-beta production	1.048×10^{-3}

version	e111_eg58_p18_b51d8f08
date	12/6/2024, 6:27:12 pm
organism	hsapiens

g:Profiler

Figure. 3:GO and KEGG enrichment analysis for common DEGs across all stages of oral cancer. *The figure shows enriched biological processes and pathways, with bar graphs depicting the significance of each term, highlighting key functional categories and pathways associated with these DEGs.*

Validation of identified potential genes in Esophageal carcinoma

To validate the potential clinical significance of the identified genes, we further investigated their differential expression in Esophageal carcinoma (ESCA) using the GEPIA platform (http://gepia.cancer-pku.cn/). Leveraging the robust datasets of TCGA and GTEx, comprising 182 tumors and 286 normal liver tissue samples, we implemented stringent criteria of absolute log2 fold change (|log2FC|)≥1 and p-value <0.01 to identify differentially expressed genes (DEGs) with high confidence.

This rigorous approach yielded a comprehensive landscape of gene expression alterations associated with OSCC progression. Tumor samples, visually distinguished by the red color on the GEPIA platform, exhibited distinct expression patterns compared to their grey-hued normal counterparts. Significant biomarkers are marked with a red star sign in the box plot of expression analysis, which is for the genes USP18, OAS2, LY6E, ISG15, IFIT3, IFI6, IFI35, and BST2. At the same time, insignificant markers are IRF7 and HERC6 (Fig. 4). This stark contrast highlights the altered transcriptional activity within the cancerous context, providing a valuable starting point for further investigation.

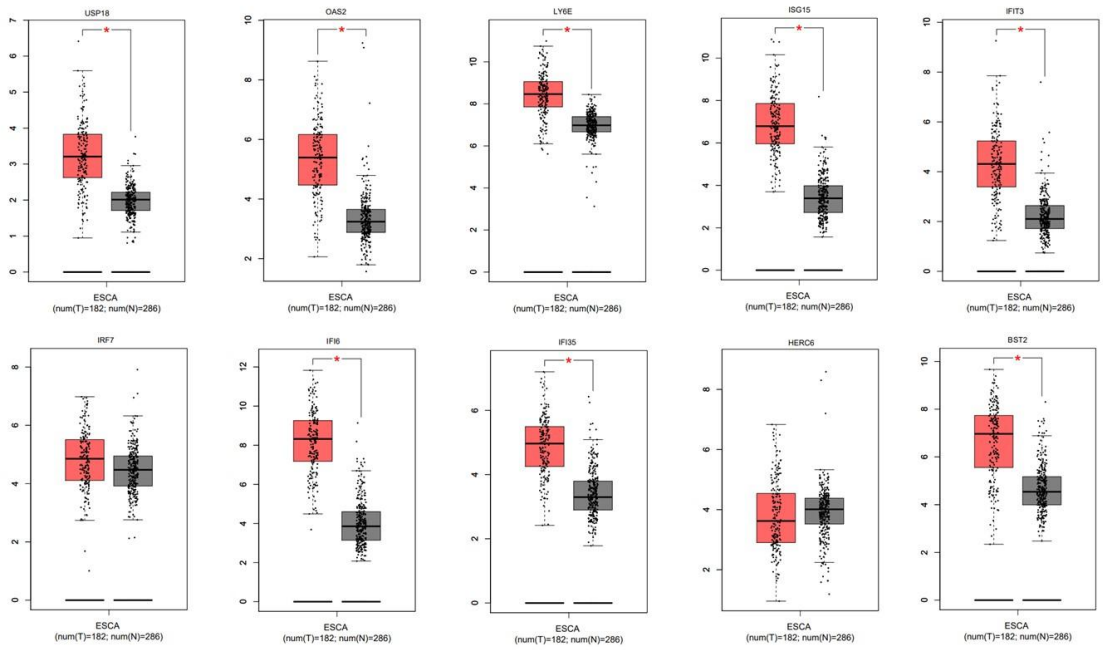

Figure 4:Box plot showing the distribution of expression levels for significant genes across different conditions or groups. *Each box represents the range and median of gene expression, highlighting differences in expression between groups and identifying outliers.*

Potential Gene-drivenSurvival Analysis

In the comprehensive survival analysis, the hazard ratios for specific genes signify their roles in the progression of diseases, with the summarized findings presented in Figure 5. Elevated expressions of USP18 (HR= 0.82) and OAS2 (HR= 0.84) correlate with increased mortality risks, underscoring their significance in cancer development, where USP18 affects interferon signaling and immune responses against tumors, while OAS2, known for its antiviral properties, also plays a role in inhibiting tumor growth. ISG15 (HR= 0.87) and IRF7 (HR= 0.95) are linked to higher mortality rates, highlighting their contributions to cancer cell proliferation; ISG15's dual role in tumorigenesis and IRF7's involvement in immune response modulation further elaborate their complex interactions in cancer dynamics. IFI6 emerges with a hazard ratio of 0.66, hinting at a protective role, possibly through its anti-apoptotic functions, which might oppose tumor development in certain contexts. The genes IFIT3 (HR= 0.94), IFI35 (HR= 0.94), and BST2 (HR= 1) show hazard ratios close to 1, indicating a more neutral prognostic value in this study. Yet, their biological functions—ranging from immune signaling to protein degradation pathways—offer avenues for further cancer-related inquiries. HERC6, with an HR of 1.2, suggests a slightly increased risk of mortality linked to its role in protein degradation pathways, which, when altered, can contribute to cancer. LY6E's expression (HR= 0.94) suggests a potential risk in cancer progression, possibly through its impact on cellular

immunity and response mechanisms. This indicates a need for further exploration in oral squamous cell carcinoma (OSCC) and other cancers Fig. 5, Table 1.

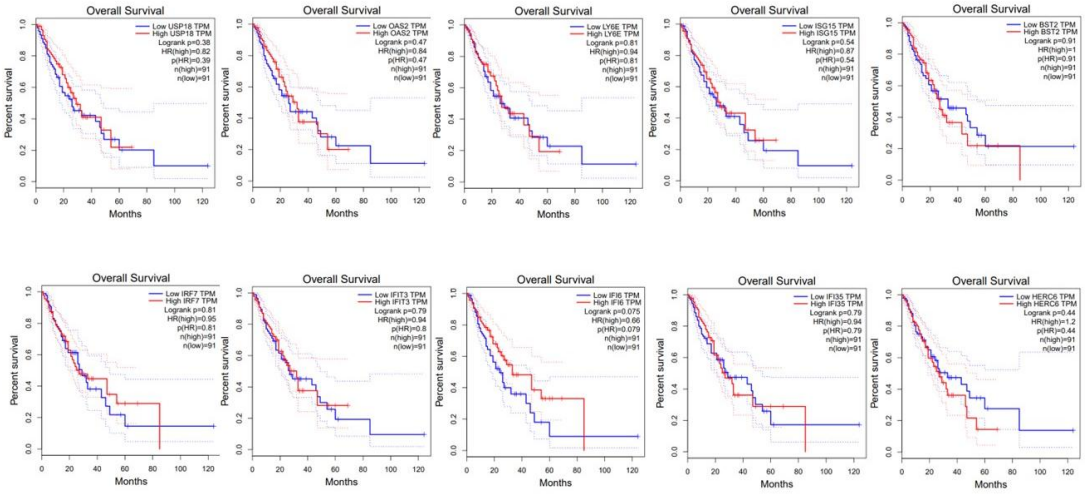

Figure 5:Survival plot illustrating the association between the expression levels of significant genes and patient survival. *The plot shows survival curves for different expression groups, highlighting how high or low expression of these genes correlates with survival outcomes.*

Gene expression analysis across cancer stages revealed distinct patterns

The violin plots are based on the Esophageal carcinoma (ESCA) data using the GEPIA platform (http://gepia.cancer-pku.cn/). Leveraging the robust datasets of TCGA and GTEx, comprising 182 tumors and 286 normal liver tissue samples, we implemented stringent criteria of absolute log2 fold change (|log2FC|)≥1 and p-value <0.01 to identify differentially expressed genes (DEGs) with high confidence. Itillustrates the distribution of gene expression levels across different cancer stages for ten genes implicated in OSCC. While some genes exhibited consistent trends, others demonstrated more complex patterns. USP18 and OAS2 displayed a progressive upregulation with advancing disease stages, suggesting their potential roles as oncogenes. Conversely, IFI6 showed a distinct pattern with higher expression in early-stage disease, hinting at a potential tumor-suppressive function. ISG15 and IRF7 exhibited more variable expression profiles, necessitating further investigation to elucidate their precise roles. The remaining genes, LY6E, IFIT3, IFI35, BST2, and HERC6, showed less pronounced expression changes across stages, suggesting their potentially limited impact on disease progression (Fig. 6, Table 1).

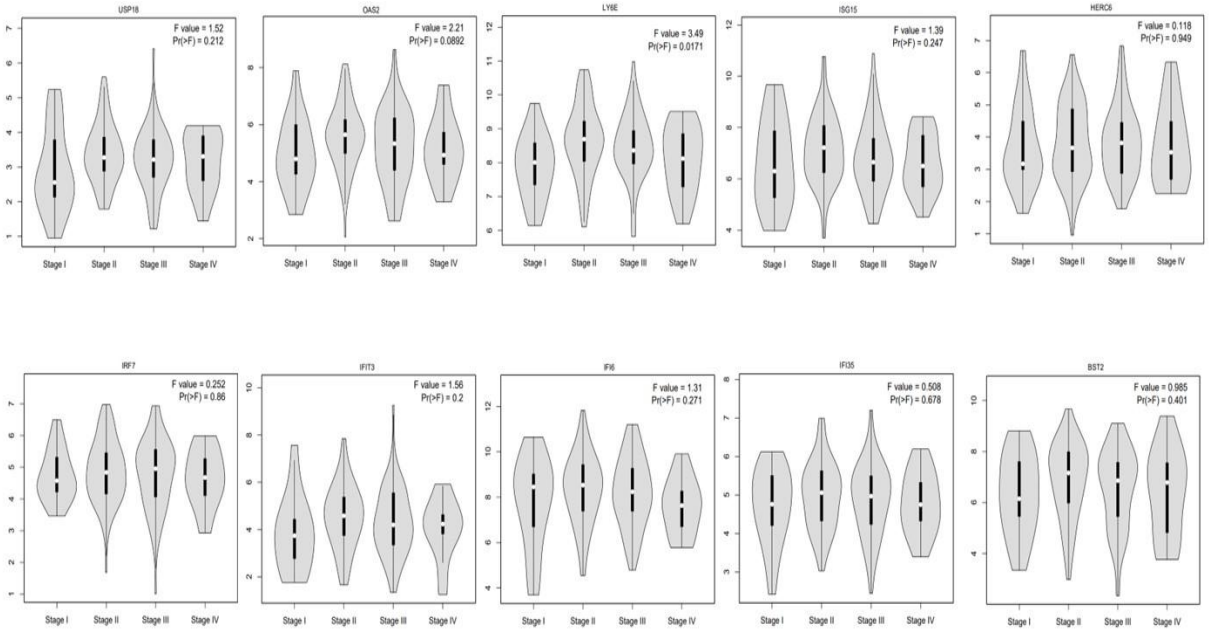

Figure 6:Violin plot displaying the distribution of expression levels for significant genes across different stages. *Each violin shape represents the density and distribution of gene expression within each stage, allowing visualization of variation and central tendencies.*

Network analysis reveals interconnectedness of key biomarkers in gallbladder cancer

Network analysis reveals the interconnectedness of key biomarkers in OSCC. Building upon the identification of potent prognostic LY6E gene in OSCC, we delved deeper into their regulatory networks using NetworkAnalyst, a powerful tool for exploring biological interactions depicted in Fig. 6. This comprehensive analysis revealed a complex interplay between transcription factors (TFs), microRNAs (miRNAs), and these key genes, shedding light on the molecular mechanisms underlying their impact on patient survival. LY6E emerged as a central hub, with interactions identified for 7 TFs, 26 miRNAs, and 1 additional gene. This extensive network suggests a multifaceted regulatory landscape where LY6E's influence on survival likely involves diverse transcriptional and post-transcriptional mechanisms.

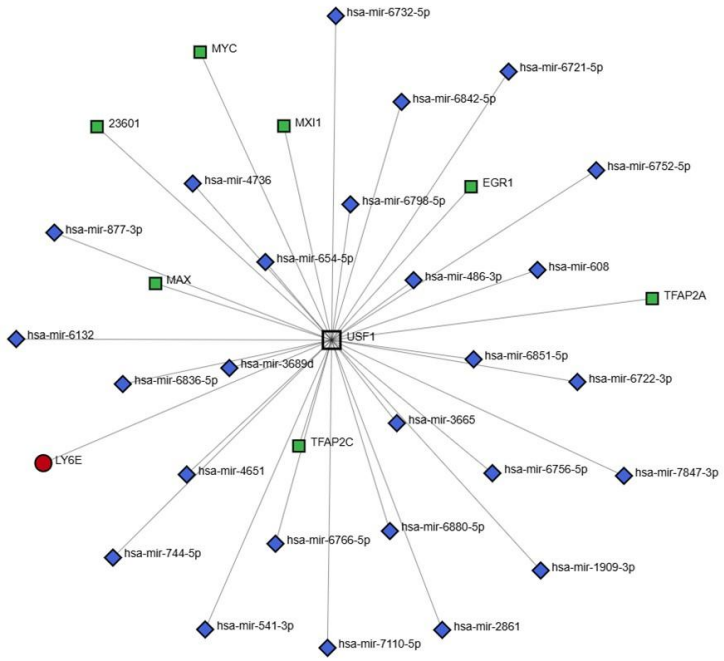

Figure 7:Prediction of miRNAs and transcription factors (TFs) for the most significant biomarker, LY6E. *The figure displays predicted miRNAs and TFs that potentially regulate LY6E, with interaction networks or lists highlighting their associations and potential impact on LY6E expression*

Discussion

The most common type of cancer, Oral Squamous Cell Carcinoma (OSCC), affects a large number of both males and females worldwide. Currently, there are very few early-stage diagnostic and prognostic biomarkers identified to improve patient outcomes. This lack of early-stage biomarkers leads to poor diagnosis and treatment options for patients. Additionally, there have been limited bioinformatics studies in the field of OSCC to identify potential biomarkers associated with its progression. Our in-depth computational analysis using various bioinformatics tools aims to uncover early-stage biomarkers linked to OSCC. In our study, we analyzed a specific dataset, GSE85195, and identified 245 DEGs (differentially expressed genes) that are crucial for the progression of OSCC. Using network-focused methods such as STRING and Cytoscape, we explored the interconnectedness of these genes and discovered a core group of 10 genes with a high MCODE score of 10. Interestingly, one gene, LY6E, was identified as a key player associated with the development and progression of OSCC. Analysis using Box Plot, Kaplan-Meier survival, and Violin Stage Plot of significant genes demonstrated that LY6E is the most significant gene, suggesting its potential as a novel prognostic biomarker for identifying, distinguishing, and predicting OSCC progression. Our

study, which employs a network-centric approach, unveils the complex interactions among differentially expressed genes in OSCC, highlighting the crucial role of LY6E in driving the disease's development and prognosis. Further understanding of this molecular interplay could lead to the design of innovative therapeutic strategies to improve patient outcomes in the fight against OSCC. Nevertheless, conducting studies on a larger patient cohort and in-depth basic mechanistic studies would provide more detailed information and implications of the LY6E gene. Our study sets the stage for future detailed investigations on LY6E as a potential biomarker for the early diagnosis of OSCC.

DECLARATION:

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Author's contributions

SBP: conceptualization and prepared the outline of the research article. RKG, RB, and HI wrote the manuscript: RB, and RKG prepared figures and tables for the manuscript. DK has done referencing and data analysis. All authors read, reviewed and approved the submitted version.

Availability of data and materials

Not applicable

Financial support and sponsorship

None

Conflicts of interest

All authors have showed no conflicts of interest.

Ethical Statements: This article does not contain any studies with human participants or animals performed by any of the authors.

Authors also declare that 'Clinical trial number: not applicable.'

Consent for publication

Not applicable.

References:

1. Tapak, L., et al., *Identification of gene profiles related to the development of oral cancer using a deep learning technique.* BMC Med Genomics, 2023. **16**(1): p. 35.
2. Reyes, M., et al., *Wnt/beta-Catenin Signaling in Oral Carcinogenesis.* Int J Mol Sci, 2020. **21**(13).
3. Dhanuthai, K., et al., *Oral cancer: A multicenter study.* Med Oral Patol Oral Cir Bucal, 2018. **23**(1): p. e23-e29.
4. Ram, H., et al., *Oral cancer: risk factors and molecular pathogenesis.* J Maxillofac Oral Surg, 2011. **10**(2): p. 132-7.
5. Dissanayaka, W.L., et al., *Clinical and histopathologic parameters in survival of oral squamous cell carcinoma.* Oral Surg Oral Med Oral Pathol Oral Radiol, 2012. **113**(4): p. 518-25.
6. Liang, K.H., et al., *Recent progress of biomarkers in oral cancers.* J Chin Med Assoc, 2021. **84**(11): p. 987-992.
7. Mascolo, M., et al., *Epigenetic disregulation in oral cancer.* Int J Mol Sci, 2012. **13**(2): p. 2331-2353.
8. Irani, S., *New Insights into Oral Cancer-Risk Factors and Prevention: A Review of Literature.* Int J Prev Med, 2020. **11**: p. 202.
9. Williams, H.K., *Molecular pathogenesis of oral squamous carcinoma.* Mol Pathol, 2000. **53**(4): p. 165-72.
10. Mertowska, P., et al., *Immunomodulatory Role of Interferons in Viral and Bacterial Infections.* Int J Mol Sci, 2023. **24**(12).
11. Chowdhary, S., et al., *Recent Updates on Viral Oncogenesis: Available Preventive and Therapeutic Entities.* Mol Pharm, 2023. **20**(8): p. 3698-3740.
12. Chen, Y., et al., *A Novel Immune-Related Gene Signature to Identify the Tumor Microenvironment and Prognose Disease Among Patients With Oral Squamous Cell Carcinoma Patients Using ssGSEA: A Bioinformatics and Biological Validation Study.* Front Immunol, 2022. **13**: p. 922195.
13. Yu, J. and S.L. Liu, *Emerging Role of LY6E in Virus-Host Interactions.* Viruses, 2019. **11**(11).
14. Javid, H., et al., *The role of viruses in cancer development versus cancer therapy: An oncological perspective.* Cancer Med, 2023. **12**(10): p. 11127-11148.
15. Usman, S., et al., *Major Molecular Signaling Pathways in Oral Cancer Associated With Therapeutic Resistance.* Front Oral Health, 2020. **1**: p. 603160.
16. Abati, S., et al., *Oral Cancer and Precancer: A Narrative Review on the Relevance of Early Diagnosis.* Int J Environ Res Public Health, 2020. **17**(24).
17. Makarev, E., et al., *In silico analysis of pathways activation landscape in oral squamous cell carcinoma and oral leukoplakia.* Cell Death Discov, 2017. **3**: p. 17022.
18. Shojaei, S., et al., *MicroRNA-Based Markers of Oral Tongue Squamous Cell Carcinoma and Buccal Squamous Cell Carcinoma: A Systems Biology Approach.* Biochem Res Int, 2023. **2023**: p. 5512894.
19. Puttaraju, M.K. and P. Nitin, *Conceptual model for progression of oral cancer - our perspective.* Am J Cancer Res, 2023. **13**(8): p. 3650-3658.
20. Cristaldi, M., et al., *Salivary Biomarkers for Oral Squamous Cell Carcinoma Diagnosis and Follow-Up: Current Status and Perspectives.* Front Physiol, 2019. **10**: p. 1476.
21. Macha, M.A., et al., *Clinical significance of TC21 overexpression in oral cancer.* J Oral Pathol Med, 2010. **39**(6): p. 477-85.

22. Quintero-Fabian, S., et al., *Role of Matrix Metalloproteinases in Angiogenesis and Cancer.* Front Oncol, 2019. **9**: p. 1370.
23. Gunardi, I., et al., *Research Trends in Molecular Biological Studies on Oral Squamous Cell Carcinoma: A Bibliometric Analysis.* Oncol Rev, 2023. **17**: p. 11585.
24. Oliveira-Costa, J.P., et al., *Gene expression patterns through oral squamous cell carcinoma development: PD-L1 expression in primary tumor and circulating tumor cells.* Oncotarget, 2015. **6**(25): p. 20902-20.
25. Wermelinger, G.F., et al., *A Novel MDM2-Binding Chalcone Induces Apoptosis of Oral Squamous Cell Carcinoma.* Biomedicines, 2023. **11**(6).