

Integrated Bioinformatics and Experimental Validation Identifies Prognostic Hub Genes in Esophageal Squamous Cell Carcinoma (ESCC)

Yalda Amiri Hezave

Hamadan University of Medical Sciences

Armin Khaghani Boroujeni

Isfahan University of Medical Sciences

Razieh Amini

Hamadan University of Medical Sciences

Ali Shojaeian

`ali.shojaeian65@gmail.com`

Hamadan University of Medical Sciences

Research Article

Keywords: squamous cell carcinoma (ESCC), biomarkers, differentially expressed genes (DEGs), hub genes, bioinformatics

Posted Date: July 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10212390/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Integrated Bioinformatics and Experimental Validation Identifies Prognostic Hub Genes in Esophageal Squamous Cell Carcinoma (ESCC)

Yalda Amiri Hezave ¹, Armin Khaghani Boroujeni ², Razieh Amini ¹, Ali Shojaeian ^{1*}

¹ Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran

² Skin Disease and Leishmaniasis Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

*** Correspondence:**

Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran, ali.shojaeian65@gmail.com, a.shojaeian@umsha.ac.ir, ORCID: orcid.org/0000-0002-1166-385X

Abstract

Background:

Esophageal squamous cell carcinoma (ESCC) is a highly aggressive malignancy with a poor prognosis and a 5-year survival rate below 20%. Late diagnosis, frequent recurrence, and therapeutic resistance are major clinical challenges. Thus, there is an urgent need to identify novel biomarkers and therapeutic targets to improve patient outcomes. **Methods:** Genes with differentially expressed genes (DEGs) in ESCC tissue against normal tissue, as well as were identified through an integrative analysis of microarray data retrieved from the Gene Expression Omnibus (GEO) database (GSE23400 and GSE157804). The STRING database was employed to construct protein–protein interaction (PPI) networks, and Kaplan–Meier plotter was utilized to identify the hub genes that were significance in prognostic. The relative expression levels of hub genes by real-time method were assessed in ESCC tissue compared to normal tissue. **Results:** Signal transducer and activator of transcription 1 (STAT1) and lysyl oxidase (LOX), were shared across microarray datasets and were associated with poor prognosis in ESCC. In concordance with Boxplot analyses, real-time PCR results revealed a significant upregulate STAT1 and LOX expression in ESCC tissue sample ($p = 0.0002$ and $p = 0.0045$, respectively) compare to non-cancerous tissue. **Conclusions:** Finally, two hub genes, STAT1 and LOX, are suitable candidates for advancing targeted therapeutic approaches for ESCC in the future. STAT1 and LOX emerge as key prognostic hub genes in ESCC, linking transcriptional regulation and ECM remodeling to tumor progression. These findings provide a foundation for further mechanistic studies and potential development of targeted therapies in ESCC.

Keywords: squamous cell carcinoma (ESCC), biomarkers, differentially expressed genes (DEGs), hub genes, bioinformatics,

1. Introduction

Esophageal squamous cell carcinoma (ESCC) is the most prevalent histological subtype of esophageal cancer, surpassing adenocarcinoma in incidence. This malignancy carries one of the poorest prognoses among all cancers, with a 5-year survival rate ranging from 10% to 20%(1). Esophageal cancer is the sixth leading cause of cancer-related mortality worldwide. Alcohol and tobacco consumption are recognized as major risk factors for ESCC. Anatomically, ESCC predominantly involves the middle and upper portions of the esophagus (2). Current standard treatment modalities for ESCC include chemotherapy, surgical resection, and radiotherapy. More recently, immunotherapy has emerged as a promising therapeutic approach for ESCC patients (3). Despite the availability of multimodal treatment strategies, ESCC remains a highly aggressive malignancy characterized by frequent recurrence, metastasis, and intrinsic heterogeneity. These features pose significant challenges to effective clinical management and are closely associated with the development of therapeutic resistance, including resistance to immunotherapy (4).

Collectively, a deeper understanding of ESCC biology, along with the development of novel diagnostic and therapeutic strategies, is urgently required to improve clinical outcomes.

A major paradigm shift in cancer therapy is the transition toward targeted therapeutic strategies. About adenocarcinoma, targeting human epidermal growth factor receptor 2 (HER2) has emerged as an established targeted therapy. However, no such targeted agent has been approved for ESCC to date(5). Therefore, the identification of reliable molecular biomarkers that can serve as potential therapeutic targets is of critical importance. In this context, the simultaneous and specific targeting of multiple dominant biological pathways may offer significant advantages, including reduced drug resistance, fewer adverse effects, and improved treatment efficacy.

Analysis of data from public repositories such as the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) provides a robust approach for identifying differentially expressed genes (DEGs) between tumor and normal tissues (6, 7). Subsequently, protein-protein interaction (PPI) network analysis serves as an effective strategy to uncover hub genes that play central regulatory roles in cancer pathogenesis (8). The identification of such key genes, particularly those associated with patient survival, may offer potential candidates for targeted therapy. Therefore, in the present study, we aimed to identify key genes in ESCC based on microarray data from the GEO database and PPI network analysis. Hub genes significantly associated with poor survival in

ESCC were selected for further evaluation. Finally, to validate our in silico findings, the expression levels of these hub genes were examined in ESCC tissue samples compared to adjacent normal margins using quantitative real-time PCR (qRT-PCR).

2. Methods

2.1. Microarray Data and DEGs

The Gene expression omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) is an open-access repository containing data obtained from high-throughput genomic hybridization data (9). The GSE157804 is a new dataset (September 10, 2023) containing expression data of lncRNAs and mRNAs from ESCC and adjacent normal tissue. Its microarray data originate from the Central Hospital of Anyang, China. The dataset includes 5 ESCC and 5 normal tissue.

The GSE23400, with the GPL96 platform, provides microarray data on September 01, 2010. This study was conducted at National Cancer Institute (NCI) in the USA. In this study, 51 ESCC tissue samples and 51 adjacent normal tissue samples were used. Two data AE43 and AE45, which were not run by HG-U133B, were excluded. Following, data analysis using R tools in GEO, considering adj P-value (FDR) <0.05 and $|\log_2\text{-fold change (FC)}| \geq 1$, differentially expressed genes were identified. The online Venn diagram tool (<https://www.interactivenn.net/>) was used to identify common DEGs between the two datasets.

2.2 Volcano Plot

Volcano plots for DEGs were generated using the online tool Huygens (<https://huygens.science.uva.nl>). The visualization of DEGs through volcano plots provides insights into the biological processes and etiology of ESCC. In generating the volcano plot, the x-axis was based on the minus $\log_{10}$ fold-change, and the y-axis was based $\log_2$ fold-change (10). This plot was generated for the DEGs of two datasets, GSE23400 and GSE157804. Subsequently, the expression levels of the top 10 genes among the DEGs were displayed in the plot.

2.3 Gene Ontology (GO)

To address the question of which biological pathways (BPs), cellular compartments (CCs), signaling pathways (SPs), and molecular functions (MFs) are most significantly involved by the DEGs, Gene Ontology (GO) enrichment analysis was employed(11). For this purpose, the g:

Profiler web server (<https://biit.cs.ut.ee/gprofiler/>) was utilized(12). Following the analysis, genes with an adjusted p-value of less than 0.05 and an intersection size greater than 10 in each pathway were selected.

2.4 PPI Network from Common DEGs

Biological processes in the body depend to interactions within protein-protein. Disruption of this protein-protein interaction (PPI) network is associated with disease. Genes located at the core of the PPI network and connections with other proteins influence signaling pathways that play a key role in disease pathogenesis(13). To determine the hub proteins implicated in esophageal carcinogenesis, a protein-protein interaction network was generated via the STRING database (<https://string-db.org>). Cytoscape is a software for visualizing and analyzing PPI networks(14). Additionally, clustering of the PPI network was performed using Molecular Complex Detection (MCODE) plugin within Cytoscape version 3.10.4. Clusters with a score greater than 3 and containing at least 10 nodes were considered significantly.

2.5 Hub Genes and Prognosis

Hub genes were identified through cytoscape analysis with node selection criteria based on degree, betweenness and closeness centrality parameters. The hub genes in ESCC were subjected to PPI network analysis. Kaplan-Meier survival analysis was also employed to evaluate the prognostic significance of these hub genes, utilizing the Kaplan-Meier Plotter online database (<https://kmplot.com/analysis>). Notably, survival curve generation was restricted to patients with ESCC. Additionally, to examine the expression of genes associated with poor prognosis in ESCC, box plot diagrams were generated using the GEPIA online database (<http://gepia.cancer-pku.cn>).

2.6 ESCC Tissue Samples and Total RNA Extraction

This study was conducted in accordance with the declaration of Helsinki and was approved by the Ethics Committee of Hamadan University of Medical Sciences (approval code: IR.UMSHA.REC.1405.004). A total of 17 pairs of ESCC and adjacent non-cancerous tissue specimens were obtained from the Tumor Bank of the Iran National Cancer Institute, Tehran, Iran. All tissues were histopathologically confirmed as ESCC or normal margins by a pathologist and immediately stored at -80°C until further processing. Total RNA was extracted from the tissues

using RNX-plus reagent (Sinaclon, Tehran, Iran) according to the manufacturer's instructions. RNA concentration and purity were assessed by measuring the absorbance ratio at 260/280 nm using a Nano Drop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was further verified by electrophoresis on a 1% agarose gel. Subsequently, synthesis of complementary DNA (cDNA) was carried out by the ultra cDNA Synthesis Kit (Pars Tous, Iran) as directed by the manufacturer from 1 µg of total RNA samples with an A260/A280 ratio ranging from 1.8 to 2.0.

2.7 Expression Analysis of Hub Genes by Real Time PCR

Real-time PCR performed by STAT1 primers: 5' TCATCAGCAAGGAGCGAGAG -3' (forward) and 5'- TCAGGGAAAGTAACAGCAGAAAG -3' (reverse) and LOX primers: 5'- ATGCCAACACCCAGAGGAG -3' (forward) and 5'- ATCATAACAGCCAGGACTCAATC -3' (reverse). 18S rRNA primers: 5'- CCATCCAATCGGTAGTAGCG-3' (forward) and 5'- GTAACCCGTTGAACCCCAT -3' (reverse) was used as reference genes. All primers were synthesized by Gene Fanavaran (Tehran, Iran).

The qPCR reaction mixture (20 µl total volume) comprised 10 µl of YTA Super SYBR® Green qPCR Master Mix 2x (Yekta Tajhiz, Iran), 0.4 µl of each forward and reverse primer, 8.2 µl of ddH₂O, and 1 µl of cDNA. This mixture was run on a LightCycler 96 Thermocycler (Roche, Mannheim, Germany) according to the kit's instructions. Following normalization to 18SrRNA as the reference gene, relative hub genes expression were determined via the $2^{-\Delta\Delta C_t}$ method. Graph Pad Prism 8 software was employed for all statistical analyses. Gene expression in ESCC were compared to non-cancerous with Student's t-test.

3. Result

3.1 Identification of DEGs

Microarray data analysis of two datasets from ESCC tissue and adjacent normal tissue, considering an FDR <0.05 and $|\log_2\text{-fold change (FC)}| \geq 1$, revealed that the GSE23400 dataset contained 537 DEGs and the GSE157804 dataset contained 2227 DEGs, with a total of 307 genes commonly shared between them (Figure 1).

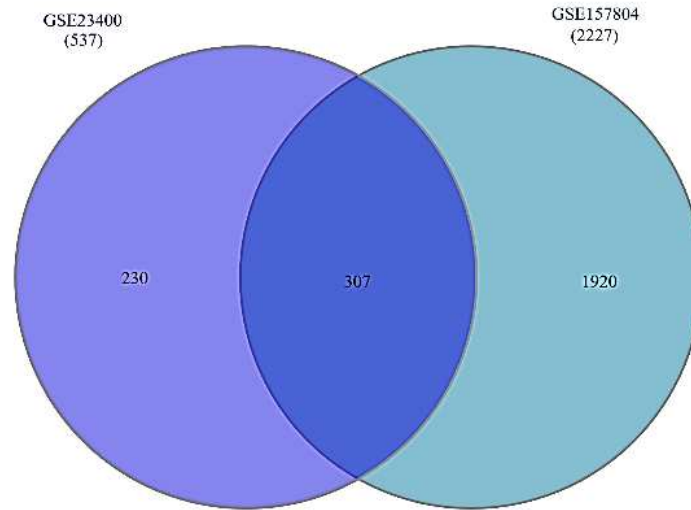


Figure 1. Venn diagram from the overlap of DEGs between two ESCC tissues from GSE23400 and GSE157804 dataset.

3.2 DEGs Volcano plot

In the volcano plot generated for the genes, the log fold change was set -1 to 1. Red dots represent upregulated genes, blue dots represent downregulated genes, and gray dots represent genes with no significant expression. The volcano plot of dataset GSE23400 showed that the upregulation of MMP1, SPP1, COL1A1, PLA1, ECT2, MFAP2, CBX3, RFC4, KIF14 and CKS1B genes plays a role in ESCC. The volcano plot of GSE157804 indicated that the upregulation of MMP1, MMP13, and CST2 genes, as well as the downregulation of THSD4, TGM3, CRISP2, CRISP3, CRNN, MAL and TMPRSS11B genes, is significant in ESCC.

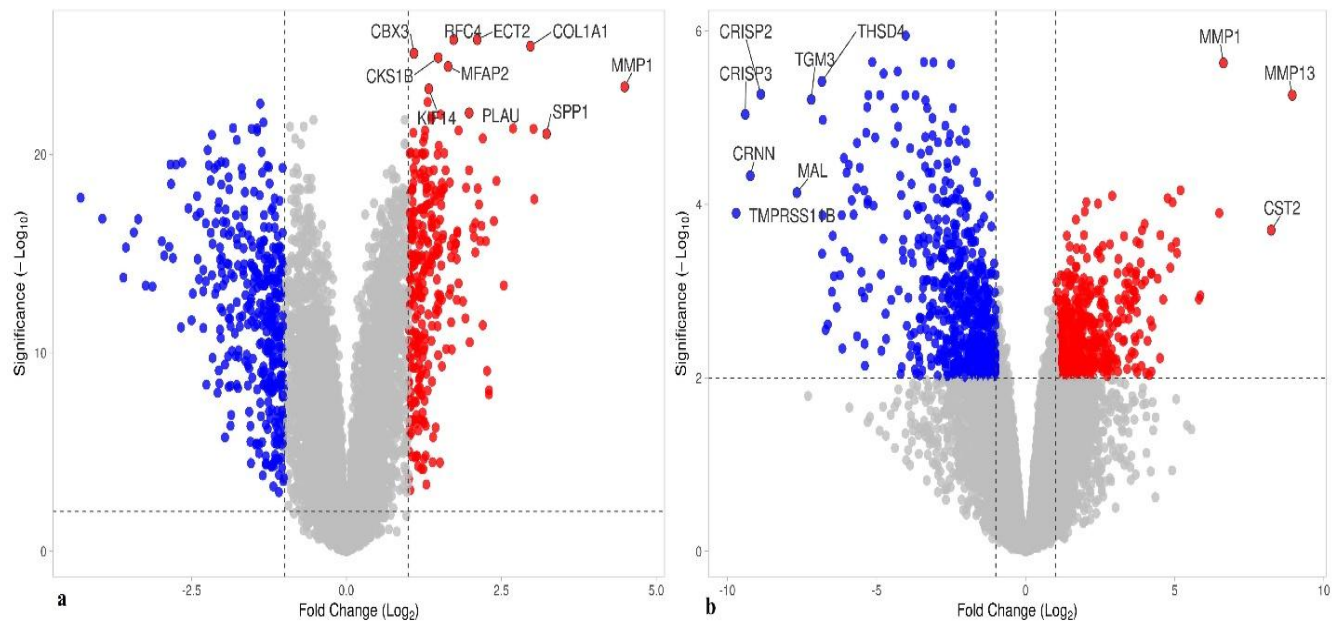


Figure 2. The volcano plot from GSE23400 (a) and GSE157804 (b) DEGs of ESCC and normal tissue.

3.3 Significant ESCC Biological Processes and Pathways

The GO enrichment analysis of BPs revealed that the DEGs were predominantly associated with cell cycle regulation and nuclear division. Notably, GO terms such as mitotic nuclear division, sister chromatid segregation and nuclear chromosome segregation exhibited the highest enrichment scores, each comprising 10 DEGs.

Analysis of SPs demonstrated significant enrichment associated with extracellular matrix (ECM) organization and cell–matrix interactions. Among the most prominently enriched pathways were Signaling by Receptor Tyrosine Kinases, Integrin cell surface interactions and ECM organization each with the highest enrichment score (Count = 16). Additionally, collagen-related pathways including Collagen formation, Collagen degradation, and Assembly of collagen fibrils were also markedly overrepresented among the DEGs (Count = 16).

Analysis of CCs demonstrated that the DEGs were predominantly localized to the extracellular space and ECM-related structures. The most significantly enriched terms included extracellular exosome, extracellular matrix, collagen-containing extracellular matrix and external encapsulating structure indicating a strong extracellular distribution of the encoded proteins.

The most significantly enriched MFs included ECM structural constituent and structural molecule activity. Predominant role of the upregulated genes in ESCC is associated with structural maintenance and remodeling of the ECM. Other enriched terms, including integrin binding, cell

adhesion molecule binding, and serine-type endopeptidase activity, were also identified, albeit with lower enrichment scores, suggesting secondary but complementary roles in cell-ECM interactions and proteolytic degradation (Figure 3).

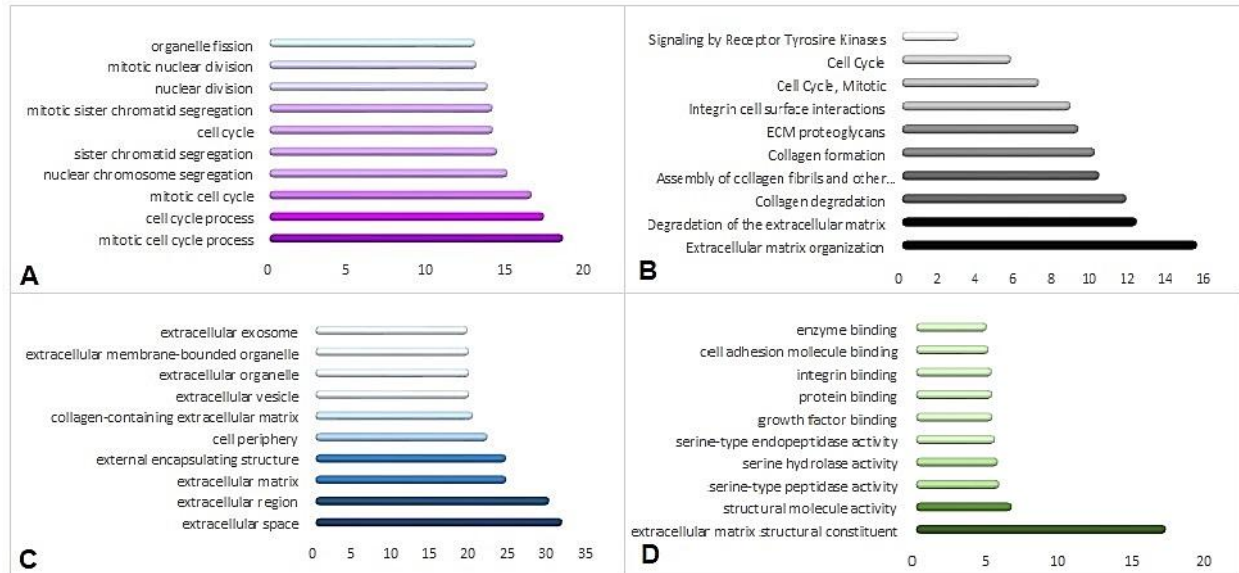


Figure 3. GO enrichment analysis of DEGs in ESCC. The bar charts represent the top enriched GO terms across four functional categories: (A) Biological Processes (B) Signaling Pathways (C) Cellular Components and (D) Molecular Functions. The count values represent the number of DEGs enriched in each GO term.

3.4 PPI Network and Key Modules

The PPI network, constructed using the STRING database, comprised 307 nodes (genes) and 1689 edges. After removing unconnected and irrelevant nodes, the refined network was visualized in cytoscape, resulting in a final network containing 269 nodes and 1689 edges. Subsequent PPI analysis using the MCODE plugin identified two significant functional modules (clusters), indicating the presence of densely interconnected gene groups within the network.

Module 1 comprised 33 genes with 508 edges and was characterized by CDK1 as the top hub gene, exhibiting the highest connectivity degree of 49. In contrast, Module 2 contained 26 genes and 264 edges, demonstrated a remarkably higher maximum degree (67) for its leading hub gene, FN1. This disparity indicates that while Module 1 represents a more distributed network of cell cycle regulators, Module 2 is heavily dominated a key ECM protein, reflecting a functional specialization toward ECM organization and integrin-mediated signaling (Figure 4).

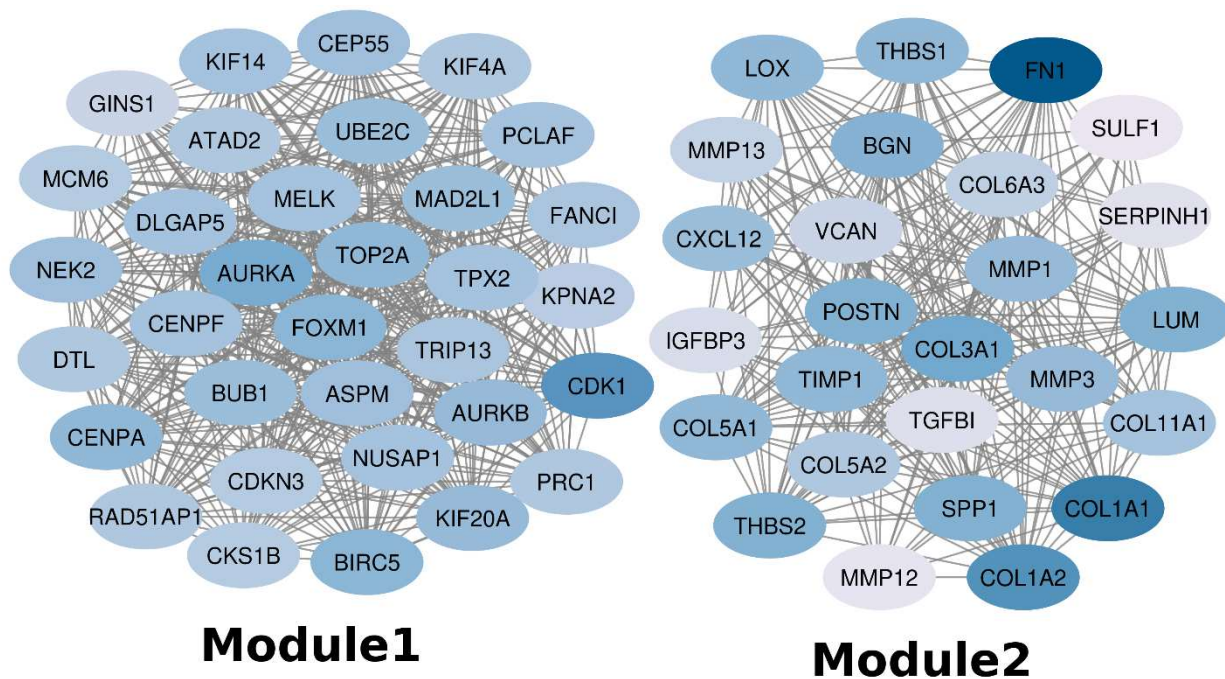


Figure 4. Identification of two significant modules from the PPI network in ESCC. Nodes are color-coded by degree centrality; CDK1 (degree = 49) and FN1 (degree = 67) represent the top hub genes in Modules 1 and 2, respectively.

3.5 Hub Genes

A total of 44 hub genes in ESCC were identified. The PPI network constructed from these 44 hub genes comprised 310 edges, indicating substantial interconnectivity among the identified hub genes (Figure 5). Survival analysis further revealed that only two of these hub genes, LOX and STAT1, were significantly associated with poor overall survival in ESCC patients (Figure 6). Specifically, LOX exhibited a hazard ratio (HR) of 2.46 (log-rank $P = 0.041$), while STAT1 demonstrated a comparable HR of 2.45 (log-rank $P = 0.045$). Box plot visualization confirmed marked upregulation of LOX and STAT1 in esophageal cancer specimens, further supporting their roles as candidate oncogenes (Figure 7).

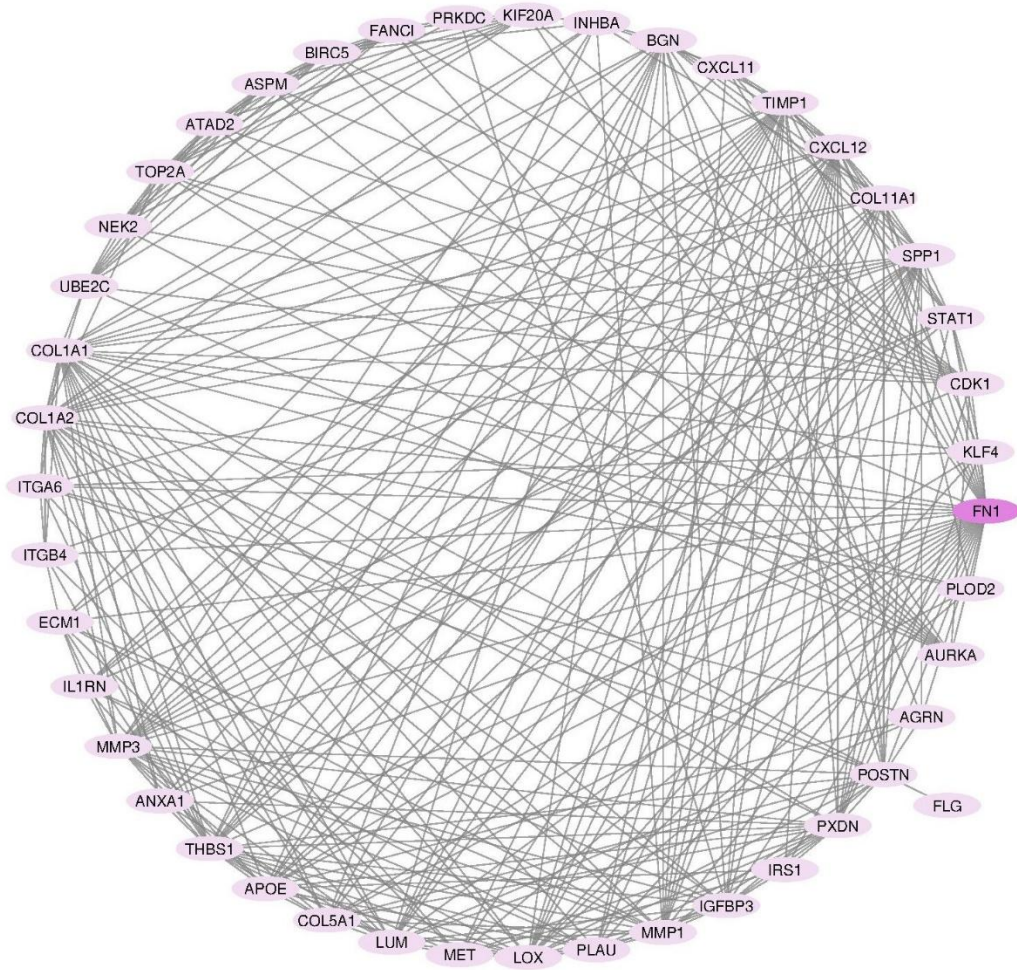


Figure 5. PPI network of 44 hub genes identified in ESCC. The network was constructed using the STRING database and visualized by Cytoscape. Fibronectin 1 (FN1) is highlighted as the node with the highest degree in the network.

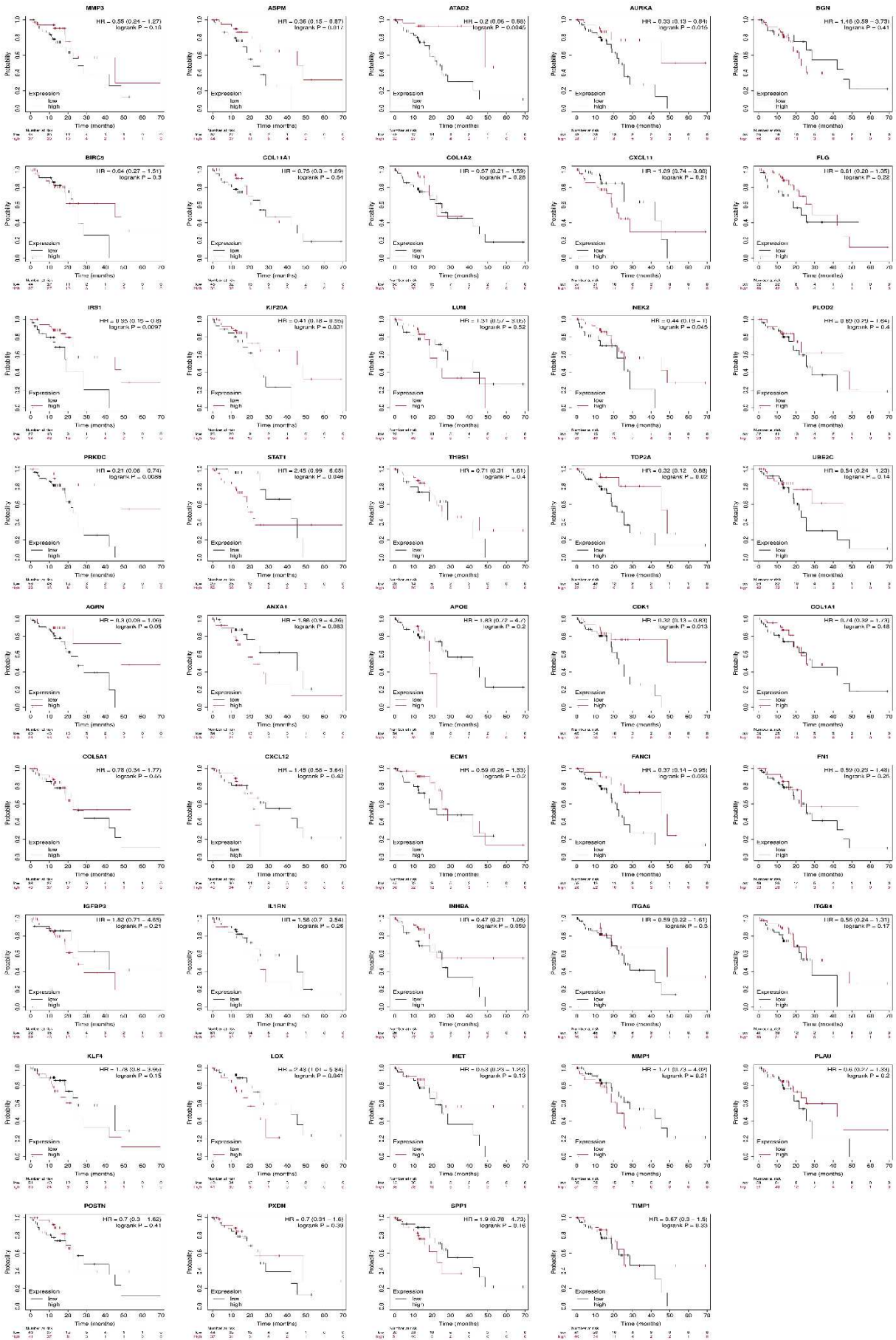


Figure 6. Kaplan-Meier survival analysis of 44 hub genes in ESCC patients using the GEPIA database.

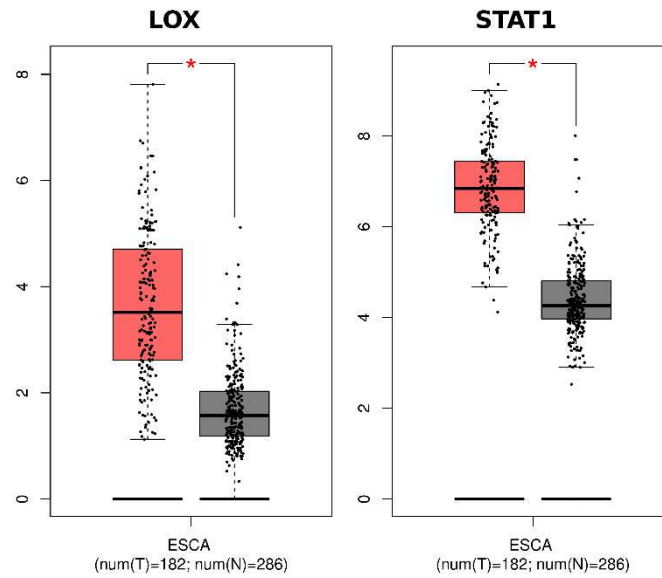


Figure 7. Box plot analysis of LOX and STAT1 expression in ESCC and normal tissues.

3.6 Hub Genes expression

To validate the bioinformatics findings, the expression levels of the hub genes (STAT1 and LOX) were examined in 17 paired ESCC tumor tissues and adjacent normal margins using qRT-PCR. The relative expression of each gene was calculated using the $2^{-\Delta\Delta C_t}$ method and normalized to 18S rRNA as the internal control. Our results revealed that both STAT1 and LOX were significantly overexpressed in ESCC tumor tissues compared to their corresponding normal counterparts. Specifically, STAT1 expression was markedly elevated in tumor samples ($p=0.0002$), and LOX also exhibited a significant increase in tumor tissues ($p=0.0045$). These findings are consistent with our in silico analysis, further confirming the upregulation of these hub genes in ESCC.

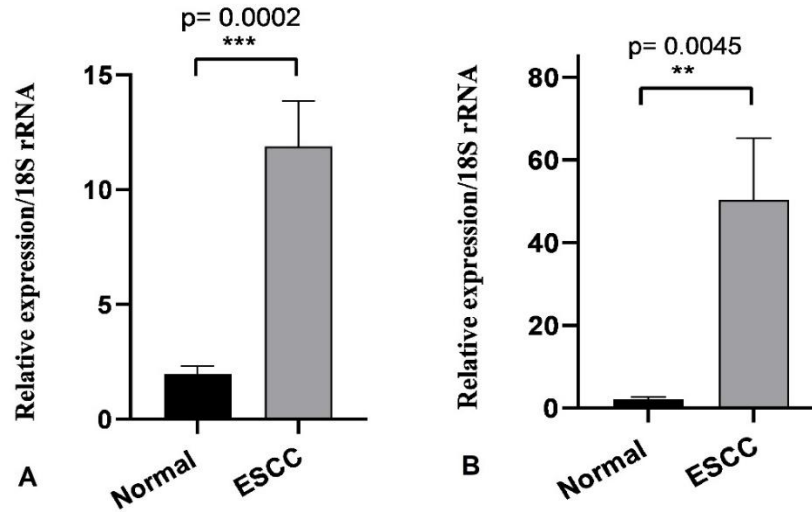


Figure 8. QRT-PCR analysis of STAT1 (A) and LOX (B) expression in ESCC tumor tissues versus normal margins, normalized to 18S rRNA. Both genes showed significantly higher expression in tumor samples ($P < 0.05$).

4. Discussion

Due to the anatomical location of the disease, the quality of life of ESCC patients is significantly compromised. Moreover, the lack of early diagnosis often results in approximately half of the patients presenting with metastasis to regional lymph nodes and other distant tissues at the time of diagnosis, which is associated with a marked reduction in overall survival (15, 16).

We employed an integrated in silico approach to identify key genes and pathways involved in ESCC pathogenesis. Our results demonstrated that both STAT1 and LOX were significantly upregulated in ESCC compare to normal tissues ($p = 0.0002$ and $p = 0.0045$, respectively), and Kaplan-Meier survival analysis revealed that elevated expression of these genes was significantly correlated with poor overall survival. GO enrichment analysis further revealed that mitotic progression and cell cycle regulation were among the most prominent BPs affected in ESCC. Additionally, SPs related to cell adhesion and ECM remodeling were significantly enriched, suggesting their critical roles in tumor cell migration and metastasis. CCs analysis confirmed that differentially expressed genes primarily influence the tumor microenvironment, while MFs analysis pointed to a predominant role in ECM remodeling.

PPI network analysis identified two significant functional modules. Module 1 was predominantly composed of genes involved in cell cycle progression, whereas Module 2 encompassed genes associated with ECM organization and tumor microenvironment remodeling. These findings

highlight the cooperative but distinct roles of these two modules in driving ESCC progression, with Module 1 driving proliferation and Module 2 facilitating invasion and metastasis.

STAT1 is a transcription factor that is activated by a wide range of interferons (IFN α , IFN β , IFN γ), interleukins (IL2, IL6, IL21, IL27) and growth factors (EGF, VEGF, and HGF). Its activity is tightly regulated at multiple levels through post-translational modifications, including phosphorylation, acetylation, and methylation, occurring at distinct residues. Following activation, STAT1 translocates to the nucleus, where it binds to specific DNA consensus sequences namely GAS (Gamma interferon-Activated Sequence) and ISRE (Interferon-Stimulated Response Element) motifs thereby modulating the transcription of diverse target genes (17). This complex regulatory network enables STAT1 to exert pleiotropic effects on various cellular processes.

Considering the intrinsic heterogeneity of tumors, STAT1 plays a dual and context-dependent role in malignancy. Elevated STAT1 expression has been linked to a favorable prognosis in ovarian and colorectal cancers, whereas its upregulation is associated with poor clinical outcomes in glioblastoma (GBM) and pancreatic ductal adenocarcinoma (PDAC). At the molecular level, STAT1 exerts its tumor-suppressive effects through transcriptional activation of genes regulating cell cycle progression, pro-apoptotic factors, and death receptors (18-20).

STAT1 exists in two isoforms, α and β . The β isoform is downregulated in esophageal cancer. This β isoform binds to the α isoform, preventing its degradation and modulating the transcriptional activity of STAT1. Loss of the β isoform is associated with increased tumor invasion and poor patient survival in ESCC (21). Studies on ESCC have revealed a dual role for STAT1, with some reports indicating a tumor-suppressive function, while others have demonstrated an oncogenic role for this transcription factor.

In this context, Findings have demonstrated that IFN- γ -induced STAT1 activation is involved in the apoptosis of ESCC cells (22). The tumor-suppressive function of STAT1 is exerted through the modulation of key regulators of apoptosis and cell-cycle progression (23).

Zhang et al. (2014) demonstrated that STAT1 expression is heterogeneous in ESCC and STAT1 exerts tumor-suppressive effects through downregulation of STAT3 and NF- κ B signaling pathways(24). Wang et al. (2017) demonstrated that STAT1 expression is downregulated in ESCC, and this reduction correlates with increased expression and activation of extracellular regulated protein kinases (ERK), a known driver of tumor progression (25)

Liu et al. (2018) demonstrated that STAT1 exerts its tumor-suppressive role in ESCC by directly inhibiting STAT3 transcriptional activity and DNA binding, and that STAT1 and STAT3 expression are inversely correlated in tumor tissues (26). Ichiba et al. (2002) showed that EGF inhibits the growth of TE8 esophageal cancer cells through STAT1-dependent induction of p21/WAF1, but this effect was observed only in one of three cell lines examined, highlighting the context-dependent nature of STAT1-mediated growth suppression(27). Chen et al. (2022) demonstrated that PD-L1 promotes EMT in esophageal cancer by suppressing the STAT1/IFIT2 signaling pathway, and IFIT2 functions as a tumor suppressor associated with better patient survival (28).

In a recent study, Chen et al. (2024) utilized single-cell transcriptomics to demonstrate that STAT1 expression is elevated in ESCC and directly binds to the LHPP promoter and represses its transcription following cause tumor progression and poor prognosis(29). Yu et al. showed that RNF168, which is amplified in 25% of esophageal cancer cases, physically associates with STAT1 in the nucleus and stabilizes it by inhibiting its poly-ubiquitination and proteasomal degradation. This stabilization leads to increased expression of JAK-STAT target genes, including IRF1 and IRF9, and promotes cancer cell proliferation, migration, and invasion. Notably, RNF168 amplification was also correlated with poor overall survival in ESCC patients(30). Wong et al., demonstrated that STAT1 is significantly overexpressed in ESCC tumors compared to normal tissues and serves as a critical downstream mediator of periostin (POSTN) signaling. They showed that POSTN, a matricellular protein abundantly expressed in the tumor microenvironment, cooperates with mutant p53 to activate STAT1, thereby promoting tumor cell invasion and metastasis. Notably, knockdown of POSTN in ESCC xenografts led to reduced STAT1 activation and decreased tumor invasion, underscoring the functional importance of the POSTN-STAT1 axis in ESCC progression(31). Notably, these findings are in complete alignment with our own observations. In our study, STAT1 was also significantly upregulated in ESCC tissues, further corroborating its potential role as a key oncogenic driver. Moreover, POSTN was identified as one of the hub genes in our PPI network analysis, specifically localized within Module 2, which was predominantly associated with ECM organization and cell adhesion. This functional annotation strongly supports the involvement of POSTN in tumor invasion and metastasis, consistent with its established role as a mediator of STAT1-driven aggressiveness in ESCC.

Lysyl oxidase (LOX) is a copper-dependent enzyme that plays a pivotal role in ECM remodeling and reorganization. This enzyme catalyzes the cross-linking of collagen and elastin through the deamination of lysine and hydroxylysine residues to aldehyde. Dysregulation of LOX activity contributes to ECM fibrosis and stiffening. Furthermore, LOX is involved in angiogenesis and inflammation during cancer progression. Evidence indicates that in cancer, the transcription factor hypoxia-inducible factor-1 α (HIF-1 α) binds to the LOX promoter and upregulates its expression, thereby activating the HIF-1 α /LOX/COL1 axis, which promotes tumor metastasis and migration. Notably, Module 2 of our PPI network analysis comprised several collagen-related hub genes, (COL11A1, COL5A1, COL1A1, COL3A1, COL6A3, COL1A2 and COL5A2) further underscoring the critical role of this gene axis in ESCC metastasis(32).

Kalikaw et al., who demonstrated that high LOX expression was observed in 62.3% of 284 ESCC patients and was significantly associated with poor overall survival. This expression correlated with aggressive tumor features, including deeper tumor invasion, lymph node metastasis, and venous invasion. Functionally, they demonstrated that LOX knockdown suppressed cell proliferation, migration, and invasion through downregulation of AKT and MMP2 (33).

In another study, Sakai et al., demonstrated that LOX expression is significantly elevated in ESCC tissues and is associated with poor prognosis, lymph node metastasis, and reduced overall and cancer-specific survival, suggesting its potential as a prognostic marker (34).

Given the elevated expression of LOX and its pivotal role in ECM remodeling in ESCC, targeting LOX may represent a novel therapeutic strategy that could potentially improve prognosis and reduce metastasis in this malignancy. However, accumulating evidence indicates that STAT1 exhibits dual and context dependent functions in cancer, necessitating a precise understanding of its mechanistic underpinnings prior to any therapeutic intervention. Consequently, LOX, along with STAT1-downstream signaling pathways, may serve as promising candidates for future targeted therapies from ESCC.

Conclusion

For the first time, the present study demonstrates that LOX and STAT1, two key hub genes in ESCC, are significantly overexpressed in the Iranian population. LOX exerts its effects primarily through remodeling the ECM and modulating the tumor microenvironment, whereas STAT1 influences intratumoral processes via transcriptional regulation of its target genes. Collectively, these two hub genes appear to coordinate both extra- and intratumoral compartments, thereby

contributing to ESCC pathogenesis. While the functional mechanisms underlying STAT1 action warrant further investigation, LOX may represent a promising candidate for targeted therapeutic intervention in patients with ESCC.

Declarations

Data availability statement

The microarray data were obtained from the GSE23400 and GSE157804 datasets available in the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). All the data of this study are included in this article. Data will be made available on request.

Ethics approval and consent to participate

The ethical considerations of this study have been approved by ethics committee at Hamadan University of Medical Sciences (Ethical code: IR.UMSHA.REC.1405.004). The study described has been conducted in line with the Declaration of Helsinki for experiments involving humans. Informed consent was obtained for experimentation with human samples. The privacy rights of human subjects have been observed.

Clinical trial number

Not applicable

Funding

This study was done with the financial support of the Hamadan University of Medical Sciences (Grant Number: 1404121911494).

CRedit authorship contribution statement

YAH: Writing – original draft, Data curation, Conceptualization. AKB: Writing – original draft, Methodology. RA: Writing – original draft and editing. AS: Writing-original draft, Review & editing, Supervision, Data curation, Conceptualization.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Wei DD, Fang JM, Wang HZ, Chen J, Kong S, Jiang Y-Y, et al. Perioperative immunotherapy for esophageal squamous cell carcinoma. *Frontiers in immunology*. 2024;15:1330785.
2. Chang L-J, Chou C-K, Mukundan A, Karmakar R, Chen T-H, Syna S, et al. Evaluation of spectral imaging for early esophageal cancer detection. *Cancers*. 2025;17(12):2049.
3. Wang C, Ju C, Du D, Zhu P, Yin J, Jia J, et al. CircNF1 modulates the progression and immune evasion of esophageal squamous cell carcinoma through dual regulation of PD-L1. *Cellular & molecular biology letters*. 2025;30(1):37.
4. Li Y, Li J, Mo W, Xu X. Changing landscape of advanced esophageal squamous cell carcinoma: Breakthroughs in systemic therapies. *Oncology Reports*. 2025;54(4):120.
5. Bonomi M, Spada D, Baiocchi GL, Celotti A, Brighenti M, Grizzi G. Targeting HER2 in gastroesophageal adenocarcinoma: molecular features and updates in clinical practice. *International Journal of Molecular Sciences*. 2024;25(7):3876.
6. Clough E, Barrett T. The gene expression omnibus database. *Statistical genomics: methods and protocols*: Springer; 2016. p. 93-110.
7. Tomczak K, Czerwińska P, Wiznerowicz M. Review The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemporary Oncology/Współczesna Onkologia*. 2015;2015(1):68-77.
8. Safari-Alighiarloo N, Taghizadeh M, Rezaei-Tavirani M, Goliaei B, Peyvandi AA. Protein-protein interaction networks (PPI) and complex diseases. *Gastroenterology and Hepatology from bed to bench*. 2014;7(1):17.
9. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic acids research*. 2002;30(1):207-10.
10. Li W. Volcano plots in analyzing differential expressions with mRNA microarrays. *Journal of bioinformatics and computational biology*. 2012;10(06):1231003.
11. Consortium GO. The gene ontology resource: 20 years and still GOing strong. *Nucleic acids research*. 2019;47(D1):D330-D8.
12. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g: Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic acids research*. 2019;47(W1):W191-W8.
13. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic acids research*. 2023;51(D1):D638-D46.
14. Smoot ME, Ono K, Ruscheinski J, Wang P-L, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics (Oxford, England)*. 2011;27(3):431-2.
15. Li Y, Ladd Z, Xiong Z, Bui-Linh C, Paiboonrungruang C, Subramaniam B, et al. Lymphatic metastasis of esophageal squamous cell carcinoma: the role of NRF2 and therapeutic strategies. *Cancers*. 2025;17(11):1853.
16. Zheng L, Li L, Xie J, Jin H, Zhu N. Six novel biomarkers for diagnosis and prognosis of esophageal squamous cell carcinoma: validated by scRNA-seq and qPCR. *Journal of Cancer*. 2021;12(3):899.
17. Najjar I, Fagard R. STAT1 and pathogens, not a friendly relationship. *Biochimie*. 2010;92(5):425-44.
18. Kamal E, Mohager SO. Dual roles of signal transducer and activator of transcription 1 (STAT1) in cancer: Expression Patterns, prognostic Significance, and immune associations across multiple tumor types. *Cureus*. 2025;17(9).
19. Meissl K, Macho-Maschler S, Müller M, Strobl B. The good and the bad faces of STAT1 in solid tumours. *Cytokine*. 2017;89:12-20.

20. Zhang J, Wang F, Liu F, Xu G. Predicting STAT1 as a prognostic marker in patients with solid cancer. *Ther Adv Med Oncol*. 2020; 12: 1758835920917558. Epub 2020/05/20. <https://doi.org/10.1177/1758835920917558> PMID: 32426049.
21. Zhang Y, Chen Y, Yun H, Liu Z, Su M, Lai R. STAT1 β enhances STAT1 function by protecting STAT1 α from degradation in esophageal squamous cell carcinoma. *Cell Death & Disease*. 2017;8(10):e3077-e.
22. Kaganoi J, Watanabe G, Okabe M, Nagatani S, Kawabe A, Shimada Y, et al. STAT1 activation-induced apoptosis of esophageal squamous cell carcinoma cells in vivo. *Annals of Surgical Oncology*. 2007;14(4):1405-15.
23. Zhang Y, Zhang Y, Yun H, Lai R, Su M. Correlation of STAT1 with apoptosis and cell-cycle markers in esophageal squamous cell carcinoma. *PloS one*. 2014;9(12):e113928.
24. Zhang Y, Molavi O, Su M, Lai R. The clinical and biological significance of STAT1 in esophageal squamous cell carcinoma. *BMC cancer*. 2014;14(1):791.
25. Wang H, Zhang Y, Yun H, Chen S, Chen Y, Liu Z. ERK expression and its correlation with STAT1 in esophageal squamous cell carcinoma. *Oncotarget*. 2017;8(28):45249.
26. Liu Z, Zhang Y, Chen Y, Lin Y, Lin Z, Wang H. STAT1 inhibits STAT3 activation in esophageal squamous cell carcinoma. *Cancer Management and Research*. 2018:6517-23.
27. Ichiba M, Miyazaki Y, Kitamura S, Kiyohara T, Shinomura Y, Matsuzawa Y. Epidermal growth factor inhibits the growth of TE8 esophageal cancer cells through the activation of STAT1. *Journal of gastroenterology*. 2002;37(7):497-503.
28. Chen J, Liu Y, Zhu Y, Chen Y, Feng J, Jiang T, et al. STAT1/IFIT2 signaling pathway is involved in PD-L1-mediated epithelial-to-mesenchymal transition in human esophageal cancer. *Clinical and Translational Oncology*. 2022;24(5):927-40.
29. Chen X, Xu Z, Zhou W, Zhang L, Huang J, Bao D. Single-Cell Transcriptomics Reveals STAT1 Drives LHPP Downregulation in Esophageal Squamous Cell Carcinoma Progression. *Technology in Cancer Research & Treatment*. 2024;23:15330338241293485.
30. Yu N, Xue M, Wang W, Xia D, Li Y, Zhou X, et al. RNF 168 facilitates proliferation and invasion of esophageal carcinoma, possibly via stabilizing STAT 1. *Journal of Cellular and Molecular Medicine*. 2019;23(2):1553-61.
31. Wong G, Lee J, Park Y, Klein-Szanto A, Waldron T, Cukierman E, et al. Periostin cooperates with mutant p53 to mediate invasion through the induction of STAT1 signaling in the esophageal tumor microenvironment. *Oncogenesis*. 2013;2(8):e59-e.
32. Farhat A, Ferns GA, Ashrafi K, Arjmand M-H. Lysyl oxidase mechanisms to mediate gastrointestinal cancer progression. *Gastrointestinal Tumors*. 2021;8(1):33-40.
33. Kalikawe R, Baba Y, Nomoto D, Okadome K, Miyake K, Eto K, et al. Lysyl oxidase impacts disease outcomes and correlates with global DNA hypomethylation in esophageal cancer. *Cancer science*. 2019;110(12):3727-37.
34. Sakai M, Kato H, Sano A, Tanaka N, Inose T, Kimura H, et al. Expression of lysyl oxidase is correlated with lymph node metastasis and poor prognosis in esophageal squamous cell carcinoma. *Annals of surgical oncology*. 2009;16(9):2494-501.