SN Computer Science (2023) 4:114
https://doi.org/10.1007/s42979-022-01492-4

ORIGINAL RESEARCH

Identification of Potential Biomarkers Using Integrative Approach: A Case Study of ESCC

Manaswita Saikia[1] · Dhruba K. Bhattacharyya[1] · Jugal K. Kalita[2]

Received: 31 May 2022 / Accepted: 3 November 2022 / Published online: 21 December 2022

Abstract

This paper presents a consensus-based approach that incorporates three microarray and three RNA-Seq methods for unbiased and integrative identification of differentially expressed genes (DEGs) as potential biomarkers for critical disease(s). The proposed method performs satisfactorily on two microarray datasets (GSE20347 and GSE23400) and one RNA-Seq dataset (GSE130078) for esophageal squamous cell carcinoma (ESCC). Based on the input dataset, our framework employs specific DE methods to detect DEGs independently. A consensus based function that first considers DEGs common to all three methods for further downstream analysis has been introduced. The consensus function employs other parameters to overcome information loss. Differential co-expression (DCE) and preservation analysis of DEGs facilitates the study of behavioral changes in interactions among DEGs under normal and diseased circumstances. Considering hub genes in biologically relevant modules and most GO and pathway enriched DEGs as candidates for potential biomarkers of ESCC, we perform further validation through biological analysis as well as literature evidence. We have identified 25 DEGs that have strong biological relevance to their respective datasets and have previous literature establishing them as potential biomarkers for ESCC. We have further identified 8 additional DEGs as probable potential biomarkers for ESCC, but recommend further in-depth analysis.

Keywords Differential expression analysis · Biomarker identification · Esophageal squamous cell carcinoma · Differentially expressed gene · RNA-sequencing · Microarray

Introduction

For a critical disease of interest, the knowledge of differentially expressed genes (DEG) are a crucial step toward biomarker identification. This is achieved through Differential Expression Analysis (DEA) which monitors the behavior of each gene in isolation over normal and disease conditions and streamlines the search for biomarkers by providing a candidate list of these discriminative candidate genes. DNA microarray and RNA Sequencing (RNA-Seq) are indispensable methods for DEA. Previously, microarray technology was the most widely used approach. However, there are inherent limitations such as the pre-requisite prior knowledge of the sequence for the array design or the fact that cross-hybridization makes it difficult to analyze highly correlated sequences. Furthermore, the lack of sensitivity to highly or lowly expressed genes as well as the lack of reproducibility across laboratories and platforms pose major challenges. These limitations are overcome by RNA-Seq technology.

Dhruba K. Bhattacharyya has contributed to this work.

This article is part of the topical collection "Pattern Recognition and Machine Learning" guest edited by Ashish Ghosh, Monidipa Das and Anwesha Law.

✉ Manaswita Saikia
saikiamanaswita@gmail.com

Dhruba K. Bhattacharyya
dkb@tezu.ernet.in

Jugal K. Kalita
jkalita@uccs.edu

[1] Department of Computer Science and Engineering, Tezpur University, Napaam, Tezpur, Assam 784028, India

[2] Department of Computer Science, College of Engineering and Applied Science, University of Colorado, Colorado Springs, CO 80918, USA

Numerous DEA methods have been developed to serve both microarray and RNA-Seq technologies. Furthermore, a large number of datasets related to critical diseases that are compatible with both technologies are widely available. Keeping in mind the fact that most methods developed for these technologies are not effective for all cases, we propose a consensus-based integrative approach that ensembles a selected few of these methods with the aim to achieve improved performance. In other words, in this paper, we present a consensus-based approach that employs a few chosen microarray DEA methods (Limma [1], SAM [2] and EBAM [3]) and RNA-Seq (Limma+Voom [4], edgeR [5], DESeq2 [6]) to present an unbiased list of DEGs as candidates for potential biomarkers. As the primary focus of our work is on the critical disease ESCC, we validate our results on two ESCC microarray datasets (GSE20347 and GSE23400) datasets and one ESCC RNA-Seq (GSE130078) dataset.

The rest of the paper is organized as follows. Section "Related Work" provides a brief overview of related work. Section "Proposed DE Framework" describes our proposed framework for biomarker identification of critical disease(s) employing three microarray and three RNA-Seq methods. We also introduce our consensus function for unbiased integration of DEGs individually detected by previously mentioned methods. Section "Analysis" reports a detailed experimental analysis and validation of our method on two benchmark microarray gene expression datasets and one RNA Sequencing (RNA-Seq) dataset. Section "Discussion" presents a detailed analysis and discussion on candidate genes that have been identified as potential biomarkers for ESCC. In this section, we also present a comparison of our algorithm with two recent works with similar approaches. Finally, the concluding remarks are given in Section "Conclusion".

Related Work

A number of statistical approaches are used by Limma [1] to achieve effectiveness in large-scale expression studies. Limma takes advantage of the flexibility of linear models and fits one to each row (gene) of data in a gene expression matrix where columns correspond to samples. Limma has the inherent ability to model correlations between samples through analysis of the entire dataset as one entity. Significance Analysis of Microarrays (SAM) [2] assimilates a set of gene-specific *t*-tests to identify genes that exhibit statistically significant changes in expression. For each gene, based on the changes in gene expression in terms of standard deviation for repeated measurements, SAM assigns a score. Potentially significant genes are identified using a threshold for these scores. Empirical Bayes Analysis of Microarrays (EBAM) [3] employs the removal Bayes rule to obtain the posterior probability that a gene was affected or unaffected under the various conditions. EBAM makes multiple testing a possibility by establishing a connection between prior probabilities and local false discovery rate (local fdr) in turn handling the issues that arise from simultaneous tests.

Voom [4] works on the idea that commonly used microarray-based statistical methods can be applied to read counts of corresponding RNA-Seq through robust and non-parametric estimation of mean-variance. In other words, Voom incorporates the mean-variance trend into the empirical Bayes procedure of Limma. edgeR [5] and DESeq2 [6] are estimations of gene-wise dispersion by conditional maximum likelihood, conditioning on the total count for the gene. edgeR effectively borrows information within genes to shrink the dispersion towards a consensus value through the use of empirical Bayes. It adapts for overdispersed data and incorporates an exact test to assess each gene. DESeq2 accurately estimates the expected dispersion value for genes of a given expression strength, which is then used to conform the gene-wise dispersion towards the predicted values . It also accounts for gene-specific variations and makes it possible to estimate fitted curves and testing even in settings with less information.

Proposed DE Framework

The proposed framework aims to work with microarray and RNA Sequencing data. We have chosen three methods that work on micro-array (Limma, SAM and EBAM) and three on RNA-Seq (Limma+voom, DESeq and EdgeR). First, both types of data require pre-processing. For microarray, pre-processing consists of the removal of unwanted and redundant information, normalization of the dataset, missing value estimation while for RNA-Seq data, we perform removal of low read counts, normalization, and transformation.

Pre-processing is followed by DE analysis that results in differentially expressed genes (DEG). For each data type, we employ a consensus function that filters out all common DEGs for each dataset. In other words, depending on the type of the input dataset, the DE analysis unit detects DEGs using three corresponding methods, followed by a consensus function that filters the DEGs common to all three methods as well as identify other relevant DEGs. Our consensus function is given by

$$\mathrm{DEGs}_{\mathrm{relevant}} = \mathrm{DEGs}_{\mathrm{common}} \cup \mathrm{DEGs}_{\mathrm{others}} \tag{1}$$

where

$$\mathrm{DEGs}_{\mathrm{common}} = \begin{cases} \mathrm{DEGs}_{\mathrm{limma}} \cap \mathrm{DEGs}_{\mathrm{SAM}} \cap \mathrm{DEGs}_{\mathrm{EBAM}}, & \text{for Microarray,} \\ \mathrm{DEGs}_{\mathrm{limma-voom}} \cap \mathrm{DEGs}_{\mathrm{edgeR}} \cap \mathrm{DEGs}_{\mathrm{DESeq2}}, & \text{for RNA- Seq,} \end{cases}$$

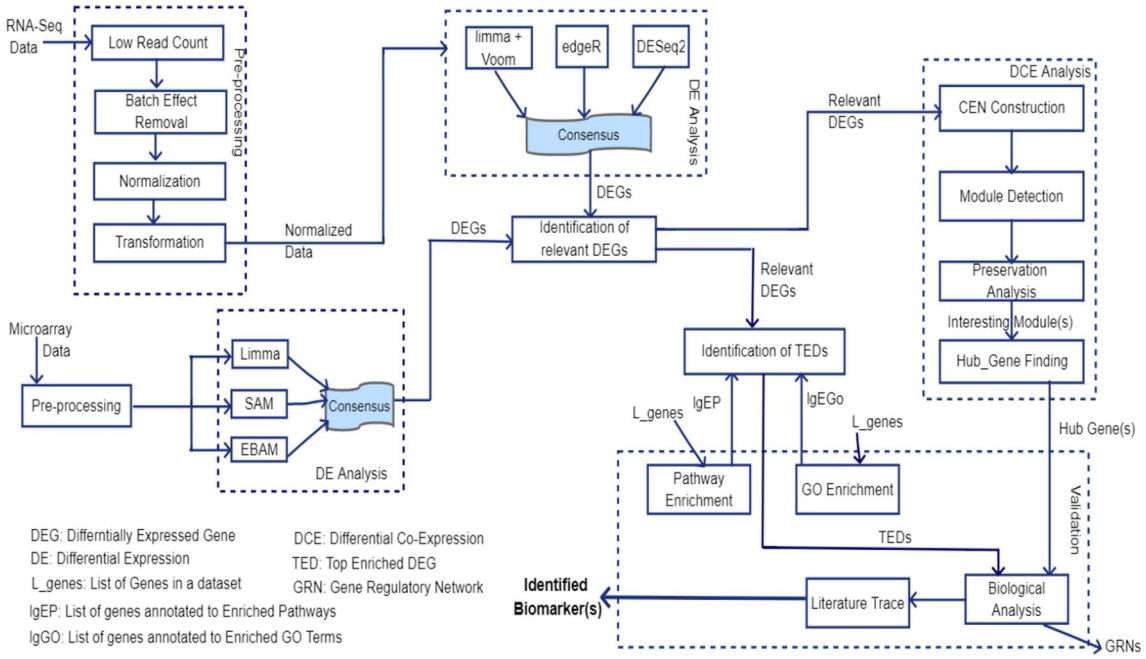


Fig. 1 Proposed framework for differential expression analysis

and

$$\text{DEGs}_{\text{others}} = \begin{cases} \text{DEGs} \notin \text{DEGs}_{\text{common}} & q\text{ - value} \leq \alpha, \text{ for RNA - Seq,} \\ \text{DEGs} \notin \text{DEGs}_{\text{common}} & local.fdr \leq \beta, \text{ for Microarray} \end{cases}$$

Here, α and β are q-value and *local.fdr* significance values that are chosen according to their relevance to the experiment. Through multiple iterations of implementation, we observed that consideration of only genes common to all three methods leads to information loss. Thus, to overcome this we introduced q-value into the consensus function. The main idea behind this is that while a p-value of 0.05 gives the implication that 5% of the tests will be false positive (FP), q-value, which is an FDR adjusted p-value, implies that 5% of the test found to be significant will be FP. q-value requires a very important adjustment for multiple tests on the same data sample. Our consensus function considers all genes common to all three methods with $p = 0.05$ as detected DEGs. Furthermore, all genes that are not among the common genes but have a $q = 0.05$, i.e. α (Eq. 1), are added to the list of DEGs. However, in the microarray datasets, we implement the proposed consensus function given by Eq. 1 to start off by taking the DEGs common to all three methods. Unlike RNA-Seq, instead of q-value, we incorporate its useful counterpart *local fdr* (β in Eq. 1). *Local fdr* is a measure of the posterior probability that the null hypothesis is true. We use *local fdr* since Limma and SAM calculate p-value. EBAM, on the other hand, estimates the posterior probability and local fdr. It is worth mentioning that posterior probability and p-value are not interchangeable. However, *local fdr* can be estimated from p-values. Our consensus function (Eq. 1) considers all genes common to all three methods with $p = 0.05$ as detected DEGs. Furthermore, all genes that are not among the common genes but have a $local.fdr = 0.05$ (β) are added to the list of DEGs.

The relevant DEGs are then taken as input to the DCE analysis unit. The idea behind performing DCE analysis is that it leads to the creation of biologically relevant modules which are easier for further analysis and validation. The DCE unit identifies differentially co-expressed modules and performs preservation analysis on these modules to identify biologically relevant modules. This is followed by the identification of hub genes in these modules using WGCNA [7] intramodular connectivity.

We validate our results using several approaches. First, we consider all relevant DEGs detected by the DE analysis unit in isolation and perform Gene Ontology (GO) and KEGG pathway enrichment analysis to validate biological relevance. We consider all the hub genes in the biologically relevant modules identified by the DCE unit as biomarker candidates. Furthermore, all DEGs that are annotated with the most enriched GO term in all three databases as well as the most enriched KEGG pathway are also considered as candidates for biomarkers. We term such genes as Top Enriched DEGs (TEDs). Secondly, we further analyze the biomarker candidates through observation of their interactions and behavioral changes. Finally, we trace literature evidence for the relevant genes in other scientific

Table 1 Datasets

Dataset	No. of genes	Normal samples	Tumor samples
GSE20347[a]	22,278	17	17
GSE23400[b]	22,283	53	53
GSE130078[c]	57,783	23	23

[a]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20347
[b]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23400
[c]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130078

sources and works for further validation of these identified genes as possible biomarkers.

Analysis

Our focus is on ESCC, a cancer very common in developing countries, especially in North-East India, and is highly attributed to tobacco and betelnut chewing, alcohol consumption as well as poor diet. Two microarray datasets GSE20347 and GSE23400 and an RNA-Sequencing dataset GSE130078 were chosen to validate our proposed DE framework (see Fig. 1). Details of each dataset is provided in Table 1. All three datasets examined gene expression in tumor and matched normal adjacent tissue. The test platform is a DELL workstation with Intel(R) Xeon(R) W-2145 with 3.70GHz processor, 64 GB RAM running Windows 10 Pro for workstations.

Preprocessing

RNA-Seq dataset GSE130078 has 57,783 genes and 46 samples. Large datasets tend to add complications to the analysis and as such, we filter out genes with low read counts. We achieve this by calculating the counts per million (CPM) for each sample for each gene and keep only those genes that have CPM > 1 for at least two samples. This reduces the dataset size from 57,783 to 22,270. We then follow up by normalization of the dataset. We also consider two microarray datasets GSE20347 and GSE23400 for analysis. The inputs to these datasets are expression values of genes across samples. First, we pre-process the data through the removal of unwanted and redundant genes, missing value estimation, and normalization. However, for both GSE20347 and GSE23400, there are no missing values and as such we proceed further down the pipeline.

DE Analysis

For the microarray datasets, Limma takes the pre-processed dataset as input and outputs the equivalent DEGs with a significance of 5% (p-value ≤ 0.05) and False Discovery Rate (FDR) of 0.05. On the other hand, for the other two methods SAM and EBAM, we employ *findDelta* with FDR $= 0.05$ fiving us an estimate of the delta values at which FDR is closest to 0.05 and chose accordingly. In SAM, delta is the distance between the observed and the expected test scores, whereas in EBAM, delta is the probability that a gene with a specific test score is differentially expressed. Table 2 summarizes the DEGs detected by all three methods on all three datasets.

In the case of the RNA-Seq dataset, the pre-processed data are the input to all three methods, i.e., Limma+Voom, edgeR and DESeq2. However, it is to be noted that while DESeq2 directly takes the count data as input, the other two methods require the count data to be transformed into a DGEList (Digital Gene Expression Data) object. All the methods perform multiple tests on all the 22,270 genes in the dataset across 46 samples. We consider a significance of 5%, i.e., p-value ≤ 0.05 and the corresponding DEGs detected by the 3 these methods are summarized in Table 2.

We implement the proposed consensus Eq. 1 to identify the common genes detected by these three methods. First, we consider the DEGs detected by all three methods, i.e. common genes. In GS20347, there are such 7706 DEGs. So as not to bypass crucial information, we use β in Eq. 1, i.e., the consensus function. With $local.fdr = 0.05$ (β) another 662 genes are considered DEGs resulting in a list of 8,368 DEGs. Similarly, in GSE23400, Limma, SAM, and EBAM find 3,431 common DEGs. With $local.fdr = 0.05$ (β), another 4,066 genes are considered as DEGs, resulting in a list of 7,497 DEGs. In the case of GSE130078, the three methods Limma+Voom, edgeR, and DESeq2 discover 2,765 common DEGs and a q-value (α) adds another 9,945 genes resulting in a list of 12,710 DEGs.

Table 2 Summary of detected DEGs by the three RNA-Seq methods and the three microarray methods

Dataset	Method	No. of DEGs with $p \leq 0.05$	Common DEGs
GSE20347	Limma	8689	7706
	SAM	10,642	
	EBAM	9565	
GSE23400	Limma	13,558	3431
	SAM	14,301	
	EBAM	3,431	
GSE130078	Limma +Voom	6858	2765
	edgeR	12,623	
	DESeq2	12,766	

DCE Analysis

To analyze the interactions among the DEGs as well as the variations in behavior under normal and disease circumstances, we construct co-expression networks (CEN) using WGCNA [7]. The pipeline for DCE analysis is to detect DEGs by our method is as follows.

1. Divide the dataset into separate datasets: All genes with only normal samples and all genes with only disease samples
2. Choose the soft thresholding power to which co-expression similarity is raised to calculate an adjacency matrix. Soft thresholding power is based on the criterion of approximate scale-free topology.
3. Construct two separate CENs in the form of an adjacency matrix: normal and disease.
4. Transform adjacency matrices into topological overlap matrices (TOM [8]) to minimize the effects of noise and spurious associations
5. Extract all connections that correspond to the subset of DEGs from both CENs.
6. Extract normal and disease modules using hierarchical clustering.
7. Merge modules through eigenvector module selection and MEDissThres threshold merging.
8. Identify modules extracted in the normal dataset that are non-preserved in disease dataset and vice versa through preservation analysis [9]. We consider such modules as modules of interest for further downstream analysis.
9. Identify the top 20 hub genes using intramodular connectivity [7] in all modules of interest.

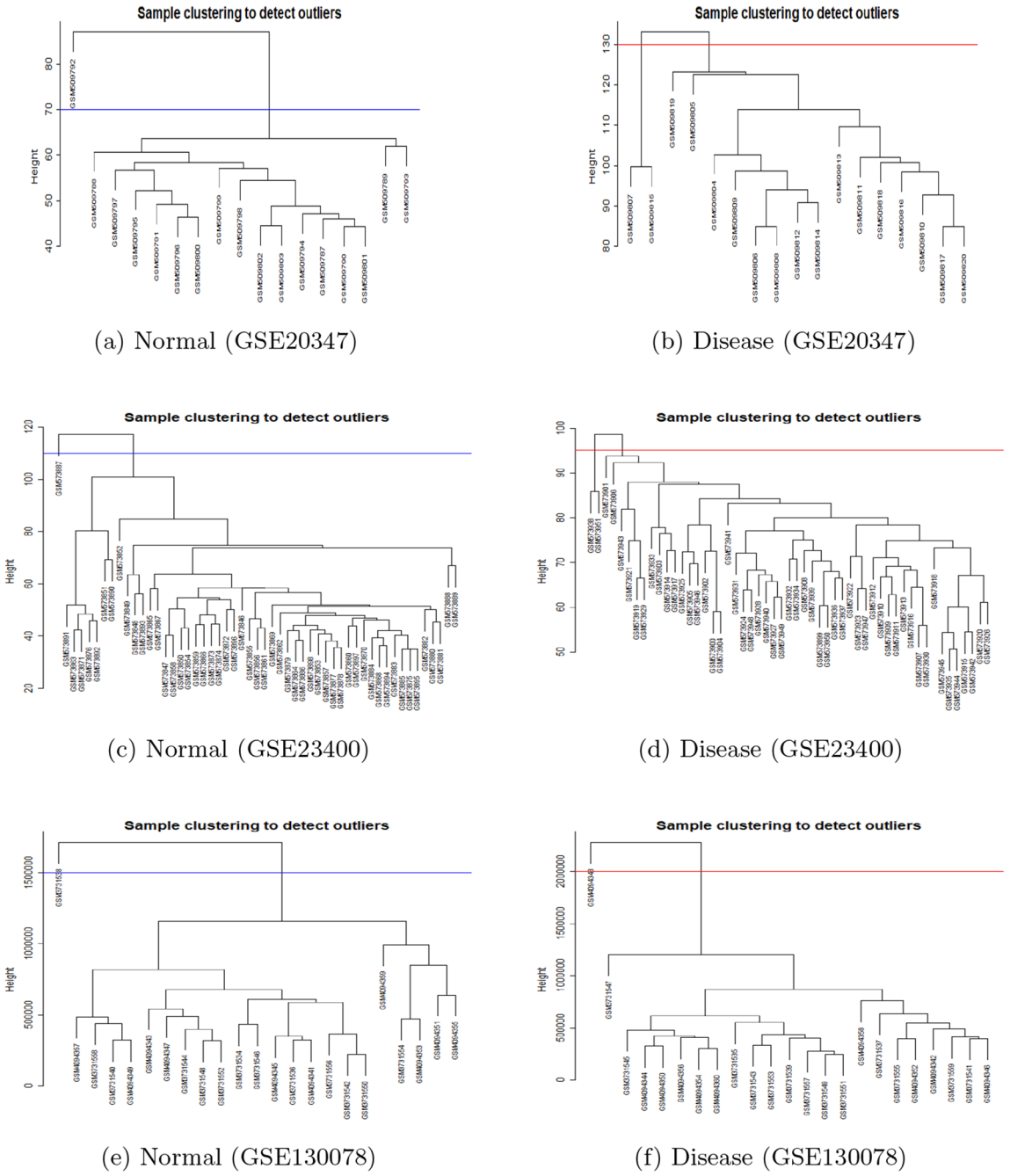
We start DCE analysis by clustering the samples using the hierarchical approach to detect outlier samples. We remove the outlier samples with the aim of creating a more robust CEN. For GSE23047, as seen in Fig. 2a, b, we find a single outlier in the case of normal samples with a tree cut at height $h = 70$ (Blue). However, in disease samples, there are two outliers with a cut at $h = 130$ (Red). Similarly, in the case of GSE23400, as seen in Fig. 2c, d, a tree cut at height $h = 105$ (Blue) and at $h = 95$ (Red) removes one and two outliers from normal and disease samples, respectively. In the case of GSE130078, a cut at $h = 1{,}500{,}000$ (Blue) and $h = 2{,}000{,}000$ (Red) removes one normal (Fig. 2e) and one disease Fig. 2f sample.

In GSE20347, hierarchical Clustering and tree cut results in 50 and 75 normal and disease modules, respectively. Figure 3a shows the dendrogram while the first strip of colors below represents the corresponding module colors for the normal dataset. Similarly, Fig. 3b shows the dendrogram for the disease dataset. To merge modules, we choose a height cut of 0.25, corresponding to a correlation of 0.75. Merging of the modules with tree cut at $h = 0.25$ further reduces the number of modules to 38 and 61 for normal and disease datasets, respectively. The second color strip in Fig. 3a, b shows the colors for the merged normal and disease modules respectively. In GSE23400, hierarchical clustering results in 9 normal (the first color strip in Fig. 3c) and 13 (the first color strip in Fig. 3d) disease modules, which are then reduced to 8 normal (the second color strip in Fig. 3c) and 11 disease (second color strip in Fig. 3d) modules after merging. Finally in GSE130078, hierarchical clustering results in 65 normal (the first color strip in Fig. 3e) and 40 disease (the first color strip in Fig. 3f) modules, which are then reduced to 21 normal (the second color strip in Fig. 3e) and 24 disease (the second color strip in Fig. 3f) modules after merging.

We follow module extraction by module preservation analysis with the aim of analyzing the distinction between preserved and non-preserved modules. According to Langfelder et al. [9], while the preserved modules retain a majority of their co-expressed connections (or edges between two genes), the same cannot be perceived from non-preserved modules. According to Langfelder et al. [9], a module with $Z_{summary} < 2$ is considered non-preserved [9]. It is noteworthy that, GSE23400 due to its inherent nature, extracts a smaller number of modules with significantly larger sizes and higher densities (Fig. 4). There are no non-preserved modules with $Z_{summary} < 2$ and most modules are either moderately preserved or highly preserved. We take into consideration moderately preserved modules with $Z_{summary} < 10$ [9].

Table 3 summarizes the preservation analysis for non-preserved modules in all three datasets. The second column highlights the module preservation reference and test networks. For example, the table reading for module *pink* in Normal/Disease subset of dataset GSE20347 can be interpreted module *pink* of size 278 detected in the normal network that is non-preserved in disease network with a $Z_{summary}$ value of – 0.118842161.

We only consider non-preserved modules of substantial size ($size > 100$) as modules of interest for further downstream analysis and validation. To find the hub genes for each module of interest extracted previously we employ WGCNA intramodular-connectivity proposed by Langfelder et al. [7]. Intramodular-connectivity calculates the connectivity of a node to other nodes in the same module.



(a) Normal (GSE20347) (b) Disease (GSE20347)

(c) Normal (GSE23400) (d) Disease (GSE23400)

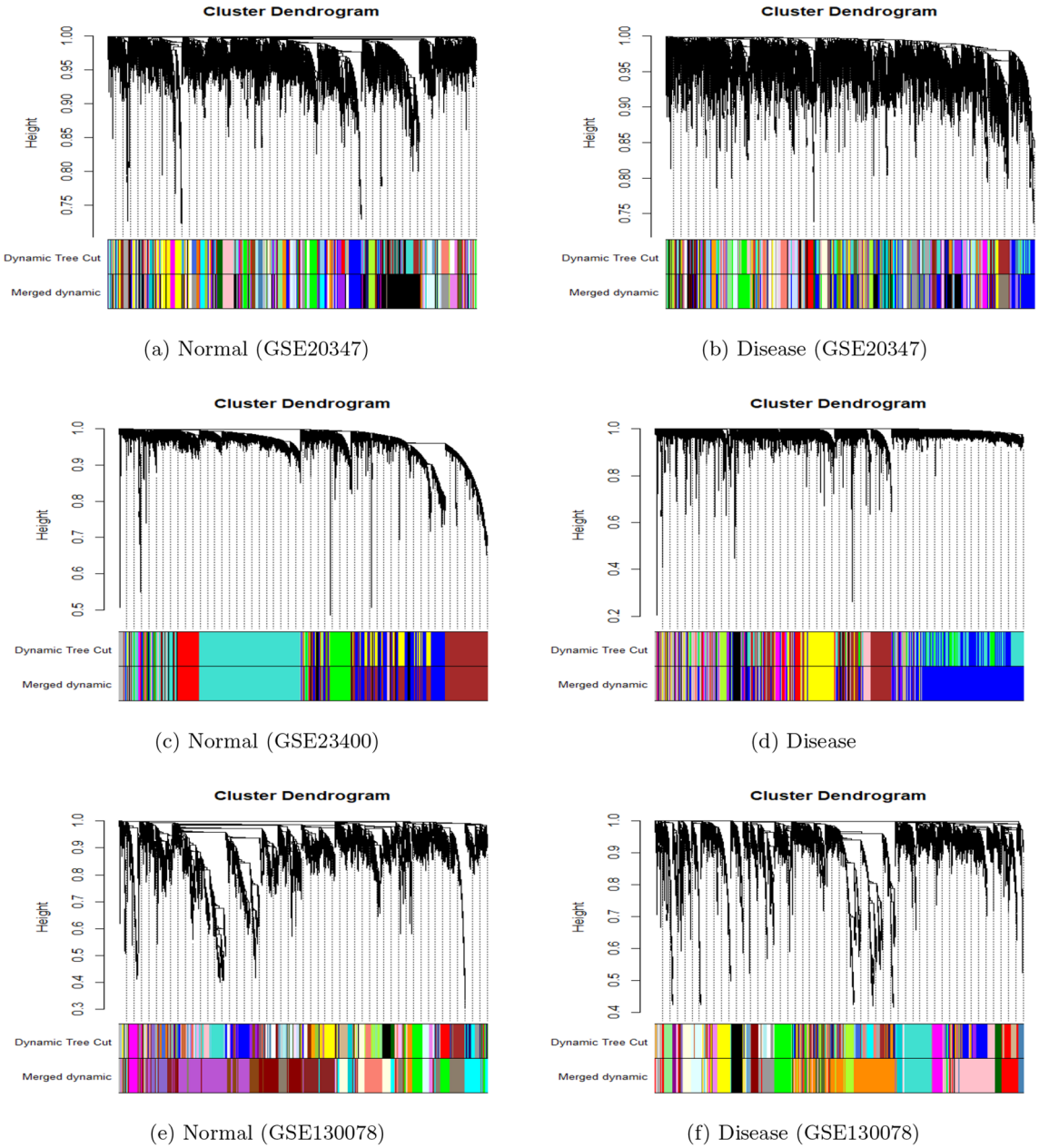
(e) Normal (GSE130078) (f) Disease (GSE130078)

Fig. 2 Outlier hierarchical trees for normal and disease samples for all three datasets

Validation

Enrichment Analysis of Modules

For a module of interest to be regarded as Gene Ontology (GO) or pathway enriched, at least one gene in the module must be assigned to an enriched GO term or pathway, respectively with a significance of 5% (i.e., $p \leq 0.05$). To perform functional enrichment analysis, we use the easily available online tool DAVID [10, 11]. Table 5 summarizes the percentages of genes in the modules of interest annotated to enriched GO terms as well as enriched KEGG pathways. We observe that all modules of interest are GO and pathway enriched.



(a) Normal (GSE20347) (b) Disease (GSE20347)

(c) Normal (GSE23400) (d) Disease

(e) Normal (GSE130078) (f) Disease (GSE130078)

Fig. 3 Dendrograms where the first strip of colors represents the colors initially assigned to corresponding modules while the second strip of colors represents the colors assigned to corresponding merged modules for the ESCC dataset: GSE20347 (**a**, **b**); GSE23400 (**c**, **d**); GSE130078 (**e**, **f**)

Candidate Genes

As mentioned earlier, we select DEGs as candidates for potential biomarkers based on the following two criteria:

1. All hub genes detected by the DCE analysis unit of our framework in all modules of interest are candidate genes.
2. DEGs that have been annotated to the most enriched GO terms in all three GO databases (BP, CC and MF) and are also annotated to the most enriched pathway after GO and Pathway enrichment analysis on the entire dataset are also considered as potential biomarkers. We rename these DEGs as TEDs (Top Enriched DEGs)

Thus, alongside all DEGs that are among Top 20 hub genes in modules of interest (as summarized in Table 4), our second criterion adds 22, 18 and 11 TEDs to the list of candidate genes in GSE20347, GSE23400 and GSE130078, respectively. We summarize these DEGs

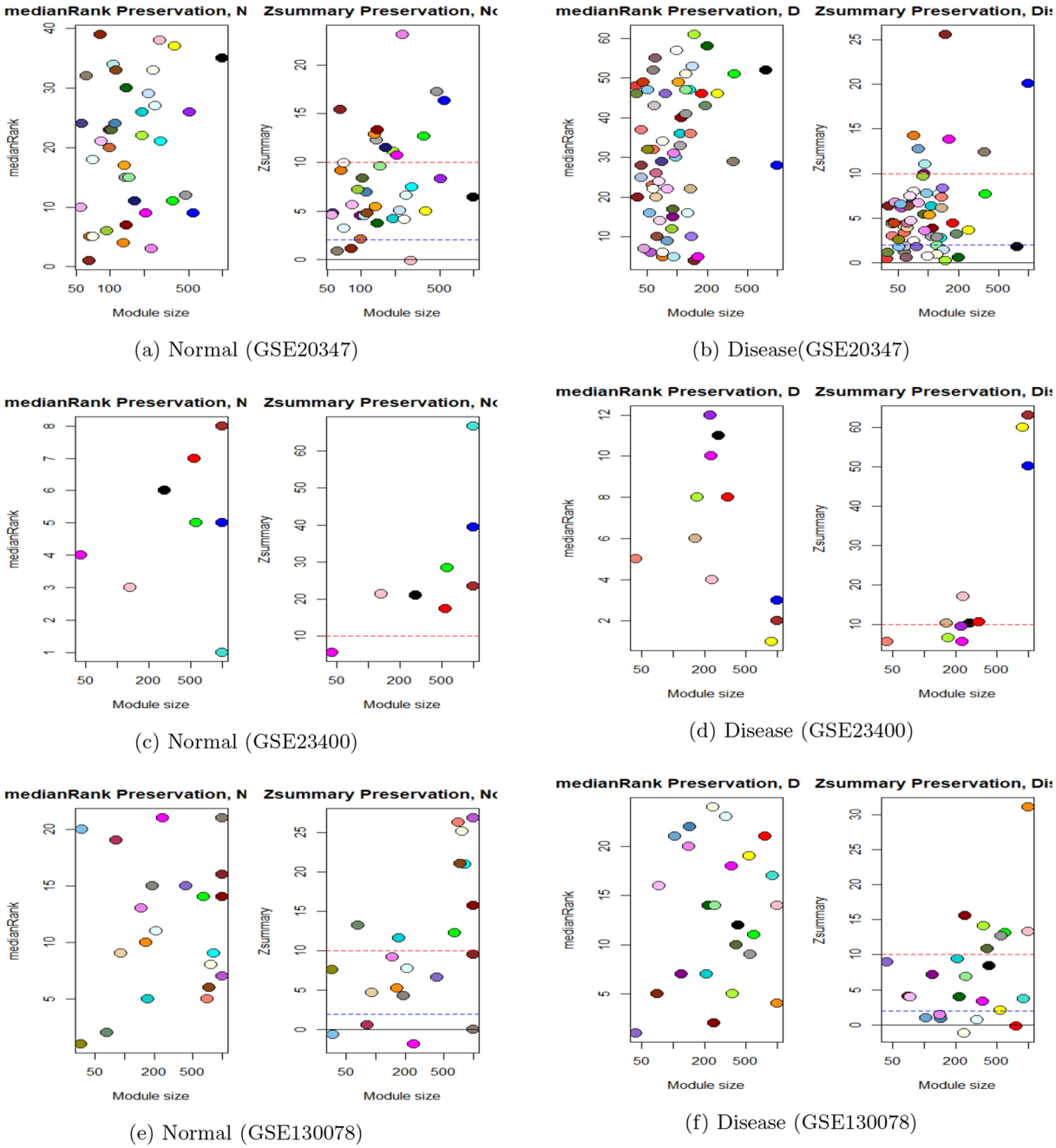


Fig. 4 $Z_{summary}$ on ESCC dataset GSE20347 (**a**, **b**); $Z_{summary}$ on ESCC dataset GSE23400 (**c**, **d**); $Z_{summary}$ on ESCC dataset GSE130078 (**e**, **f**). All modules below the red line ($Z_{summary} < 2$) are non-preserved, all modules between the red and blue lines ($2 < Z_{summary} < 10$) are weak to moderately preserved and all modules above the blue line ($Z_{summary} > 10$) have strong evidence of being preserved

(TEDs) in Table 6. The numbers of candidate genes for GSE20347,GSE23400 and GSE130078 increase from 140, 60 and 160 to 162, 78 and 171, respectively. We perform the enrichment analysis on the entire dataset or in more specific terms the list of all genes in the dataset. This leads to the observation that as the lists of genes in GSE20347 (22,278 genes) and GSE23400 (22,283 genes) are almost the same, the list of top enriched genes (35 genes) extracted are the same. However, the differences in TEDs are seen (Table 6) due to the fact that there are DEGs identified in one dataset that might not be detected in the other.

Table 3 Preservation analysis of modules in all three datasets

	Ref/Test	Module	Size	$Z_{summary}$
GSE20347	Normal/Disease	*pink*	276	– 0.118842161
		bisque4	62	0.84895892
		orangered4	82	1.10844007
		grey	17	1.38809779
	Disease/Normal	*grey*	3	– 0.11691952
		greenyellow	149	0.21296495
		brown2	39	0.40355693
		darkgreen	201	0.57638054
		lightpink4	61	0.58347786
		white	99	0.72904092
		lightyellow	122	0.88046344
		darkolivegreen4	40	1.13783059
		antiquewhite4	58	1.19766058
		lightsteelblue1	143	1.42400619
		mediumpurple3	77	1.74769518
		black	775	1.79866799
		skyblue1	51	1.79952166
		lavenderblush3	60	1.83004964
		lightgreen	123	1.88961457
GSE23400	Normal/Disease	*magenta*	45	5.63609823
	Disease/Normal	*magenta*	231	5.59355067
		salmon	44	5.64755802
		greenyellow	172	6.47180995
		purple	225	9.42312232
GSE130078	Normal/Disease	*magenta*	248	– 1.80627625
		skyblue2	37	– 0.62266325
		bisque4	1000	0.01985689
		maroon	82	0.58940588
		grey	70	1.90096828
	Disease/Normal	*lightyellow*	240	– 1.18378716
		red	759	– 0.18883575
		lightcyan	321	0.68868388
		steelblue	145	0.92648735
		skyblue3	104	1.01960350
		violet	142	1.38497020

Biological Analysis

To establish the biological relevance of the candidate genes detected by our method, we use functional enrichment analysis and the construction of a gene regulatory network (GRN). Transcription Factors (TF) have remarkable diversity as well potency as drivers of cell transformation. Bhagwat et al. [12] justify the continued pursuit of TFs as potential biomarkers across many forms cancer by the prevalent deregulation of the same. We observe that 26 (hub genes:21, TEDs:5), 11 (hub genes:6, TEDs:5) and 23 (hub genes:23, TEDs:0) candidate genes detected by our method in GSE20347, GSE23400 and GSE130078, respectively are TFs. These TFs exhibit regulatory behavior in their respective modules, establishing their biological relevance. For easy visualization, we extract a manageable subset of hub genes from the non-preserved modules detected by our method (Figs. 5a–f and 6a–f). We construct a Gene Regulatory Network (GRN) with these hub genes and associated Transcription Factors (TFs) so as to observe the regulatory behavior of the corresponding genes. The resulting GRN is in the form of an adjacency list with weighted directed edges from TFs to other target genes (TGs).

As in the the case of validation of modules, we employ DAVID [10, 11] to perform functional enrichment analysis of all candidate genes detected by our method. A candidate gene can be regarded as GO enriched considering a GO database (GO_BP, GO_CC, GO_MF) if it is annotated to at least one GO term in that database with significance of 5% ($p \leq 0.05$). Tables 7, 8 and 9 summarize the candidate genes annotated to the top 3 GO terms in each GO database in GSE20347, GSE23400 and GSE130078, respectively. Similarly, a candidate gene is KEGG pathway enriched if it is annotated to at least one KEGG pathway term with significance of 5%. Table 10 summarizes the candidate genes annotated to top 3 enriched KEGG pathways in GSE20347, GSE23400 and GSE130078.

Literature Trace

Zhu et al. [13] highlight that prothymosin alpha (*PTMA*) expression was up-regulated in ESCC tissues, thus presenting *PTMA* as a potential candidate for ESCC. Tang et al. [14] indicated that the expression of PTPRF interacting protein alpha 1 is significantly increased and is related to some malignant clinical features and poor outcomes in ESCC patients, thus establishing it as a valuable biomarker for early detection, treatment formulation and prognosis evaluation for ESCC. Jiang et al. [15] suggested that downregulation of *VGLL4* was very important in the progression of ESCC, and restoring the function of *VGLL4* might be a promising therapeutic strategy for ESCC. In Shen et al. [16], homer scaffolding protein 3 (*HOMER3*) is one of the three genes presented as candidate cancer-associated genes and may play a tumorigenic role in ESCC. Ma et al. [17] summarized that upregulation of Proteasome 26S subunit non-ATPase 4 (*PSMD4*) promotes the progression of ESCC through the reduction of ERS-induced cell apoptosis. Chen et al. [18] found that

Table 4 Top 20 hub genes for each extracted module of interest in all three datasets using WGCNA [7] intramodular connectivity

Dataset	Module	Hub genes
GSE20347	*pink*	*PTMA, MED1, TRIO, TERF1,BRD2, PWP1, HSD17B10, PPFIA1, EEF1B2, ZNF148, TCOF1 NSD2, SLC25A36, RUFY3, PIK3CB, VGLL4, LYN, DDX24, EPB41L1.*
	greenyellow	*HOMER3, SHC1, EXT2, PSMD4, CLIC4, MAP3K20, DNMT3B, TGFB2, SELENOP, PSMD11, EXOSC4, SARS1, NABP1, ENTPD7, MYO1B, RAB8B, PSAT1.*
	darkgreen	*SLC3A2, IMP4, MAPRE1, RALY, PSMB5, UQCRC2, NONO, GNB5, TFRC, GNAPDA1, ODF2, NMD3, RPL22 NEU1, SENP5, NID1, ITSN2, ABI2.*
	lightyellow	*ANP32E, NEB, AHDC1, RPRM, HOXC11, ENOX2, TNS1, MAN1C1, RCN1, CNPY2, APOOL, HAUSS, SBF1, ESF1, GNAQ, LSS, MCL1.*
	lightsteelblue1	*DBF4, POP7, MCM7, RFC2, DUS4L, POM121, ZKSCAN5, ORC3, PUS7, GMCL2, PSMC2, ITPKC, TRRAP, TIMELESS, EPHA2, CRYBG2, POM121C, CEP290.*
	black	*KPNA2, RRP7A, EBNA1BP2, KIF4A, TMEM97, CYP3A5, CCT4, CKS2, HAUS7, CIAPIN1, RANBP1, PITX1, PRMT1, PNO1, MAGOHB, JPT2, SPAG5, VPS13D.*
	lightgreen	*ITGB7, CXCR3, HPRT1, TARP, NPIPB3, CD48, NEDD4L, CASP10, TP63, UBA7, ITM2A, CD3D, MSRA, ECHDC2, LST1, CD2, UBASH3A, CD52.*
GSE23400	*greenyellow*	*TAP1, PSMB9, IFIH1, HLA-F, IFIT3, HLA-G, HLA-J, IFI44L, UBE2L6, HLA-C, IFI35, CXCL10, OAS3, IFIT1, PSMB8, ISG15, GZMB, SCO2, CXCL11.*
	magenta	*CDKN3, PHB, MTHFD1, DLGAP5, EIF2S1, ZNRD2, MNAT1, TIMM9, VRK1, YIF1A, PSMA3, NASP, SRM, PSMC1, EBNA1BP2, C12orf29, GLRX5, PLEK2, TUBG1, TIMM10.*
	purple	*FCER1G, HNMT, CD14, CD163, TYROBP, LAPTM5, C1QB, MS4A4A, PLXNC1, C1QA, ENTPD1, SRGN, CD53, TFEC, ITGB2, CD86, MS4A6A, FCGR2A, C3AR1, MNDA.*
GSE130078	*lightyellow*	*PCNX1, CAV1, RRAS2, IGF2BP2, CAVIN1, PI4K2A, PPP4R4, HRH1, SAMD4A, VEGFC, FJX1, SGPP1, LINC01998, PGF, LINC02454, HIF1A, ANO4, FOLR3, FEZ1, CSF2.*
	red	*COA6, GNA13, LIN52, POLR2D, APPBP2, PPP2R5A, PPP6C, RIT1, RBBP5, MEGF9, RALB, MEF2A, ERCC3, CDC42SE1, SDE2, STARD7, CTDSPL2, BLOC1S2, DDX59, COQ10B.*
	lightcyan	*ANKRD20A8P, LINC01287, SOHLH1, CDH22, DHRS2, CRLF1, TENM1, EMILIN3, ADGRL3, AGGF1P8, CCDC144NL, RHBDL1, HCG23, LOC105370792, ADAMTS20, RPL31P25, RBMS3, TESMIN, OR11J2P, NFIB.*
	steelblue	*GMNN, RFC5, TMTC1, UBE2T, LIMK1, OSR2, CLUAP1, HMGB3, DTL, DNA2, LMO4, SENP1, ZNF367, CDK4, EXO1, MSH2, SUMO3, ARL4A, H1-2, TMEM270.*
	skyblue3	*MANEAL, CCT2, PCSK1, GNS, ZNF737, ZNF85, PANK2, NAT8B, TBK1, TBC1D15, SYT15, MON2, CXCL13, ZNF91, TDRD1, NEXMIF, TMBIM4, DLGAP1-AS5, RHOXF1-AS1, MUCL1.*
	violet	*GRID2IP, ZNF568, ZNF239, PIWIL1, HPDL, ZNF233, ELP6, ZNF470, MST1L, ZNF232, ZNF790-AS1, LRP6, ESRRG, CFAP91, ZNF829, THUMPD3, GSE1, LINC01205, ZNF667, KIF15.*
	magenta	*FAM155A, SORCS1, IGFN1, ZAN, ACAN, XIRP1, CACNA1B, DNAH10, EPHA3, CDH4, PCDH10, CACNA1E, RNF112, ST6GALNAC5, TGM4, DSCAM, CFAP61, CDH23, FNDC1, KCNH3.*
	bisque4	*CHPF, TRAM2, IGFBP3, CXCL16, PIGT, CRELD2, SEPTIN9, MFHAS1, TOR3A, PDIA4, CARMIL1, MOGS, ORAI2, CLPTM1L, ARSB, CHST15, ARFGAP1, ST6GAL1, CDK18, CSF2RB.*

Hub genes with strong literature evidence of association to esophageal squamous cell carcinoma (ESCC) are marked in Red while hub genes with evidence of association with five other SCCs namely, Oral, Tongue, Head and Neck, Tongue or Laryngeal squamous cell carcinoma are marked in Blue

overexpression of DNA methyltransferase 3b (*DNMT3b*) is responsible for more aggressive tumor growth and resistance to treatment in ESCC and is linked to activated STAT3 signaling. Liu et al. [19] concluded that Phosphoserine Aminotransferase 1 (*PSAT1*) expression was elevated in ESCC tissues compared to normal esophageal tissues and increase in the same is significantly associated with stage of disease, lymph node metastasis, distant metastasis and poor prognosis. Findings by Cheng et al. [20] suggested that through activation of the Akt and Erk1/2 signaling pathways, Non-POU Domain Containing Octamer Binding (*NONO*) plays a potent role in multiple biological aspects of ESCC. Wada et al. [21] highlighted the clinically important implications associated with Transferrin Receptor (*TFRC*) and concluded that it offers an independent prognostic factor. By employing Cox regression He et al. [22] demonstrated the prognostic value of Canopy FGF Signaling Regulator 2 (*CNPY2*) for ESCC. Yu et al. [23] demonstrated that Myeloid cell leukemia 1 (*MCL-1*) contributes to the development of ESCC. Yang et al. [24] concluded that a lower expression of Processing Of Precursor 7 (*POP7*) predicts a worse prognosis in esophageal cancer. Qiu et al. [25] suggested that through activation of AKT1/mTOR signaling pathway, maintenance complex component 7 (*MCM7*) promotes tumor cell proliferation, colony formation and migration of ESCC cells. Choy et al. [26] and [27] further suggested *MCM7* as a more sensitive proliferation markers for evaluation and for predicting various clinical outcomes of ESCC respectively. Miyazaki et al. [28] concluded that ephrin receptor A2 (*EphA2*) overexpression appears to be related to poor degree of tumor

Table 5 Percentages of genes in each module that are annotated to the Gene Ontology (GO) databases (BP: Biological Processes, CC: Cellular components or MF: Molecular function) and KEGG pathways

Dataset	Module	Size	GO_BP (%)	GO_MF (%)	GO_CC (%)	KEGG (%)
GSE130078	*lightyellow*	240	78.9	80.5	78.9	38.3
	red	759	83.3	87.6	84.8	38.9
	skyblue3	104	82.6	87.2	85.3	46.8
	steelblue	145	73.1	76.6	71.9	26.9
	violet	142	82.0	84.7	80.0	37.3
	lightcyan	321	69.1	70.7	69.4	27.7
	bisque4	1000	87.5	91.4	89.1	40.5
	magenta	249	84.4	89.9	84.9	31.0
GSE20347	*pink*	276	90.1	96.0	95.2	47.2
	greenyellow	149	98.4	98.4	97.7	49.2
	darkgreen	201	95.6	96.7	95.6	55.0
	lightyellow	122	95.0	96.0	97.0	53.5
	lightsteelblue1	143	95.2	94.4	94.4	49.2
	black	775	94.9	97.0	96.1	52.9
	lightgreen	123	90.7	98.1	92.6	56.5
GSE23400	*greenyellow*	172	97.7	98.3	96.5	63.4
	magenta	231	94.8	96.5	93.4	44.5
	purple	225	96.6	98.1	96.3	58.2

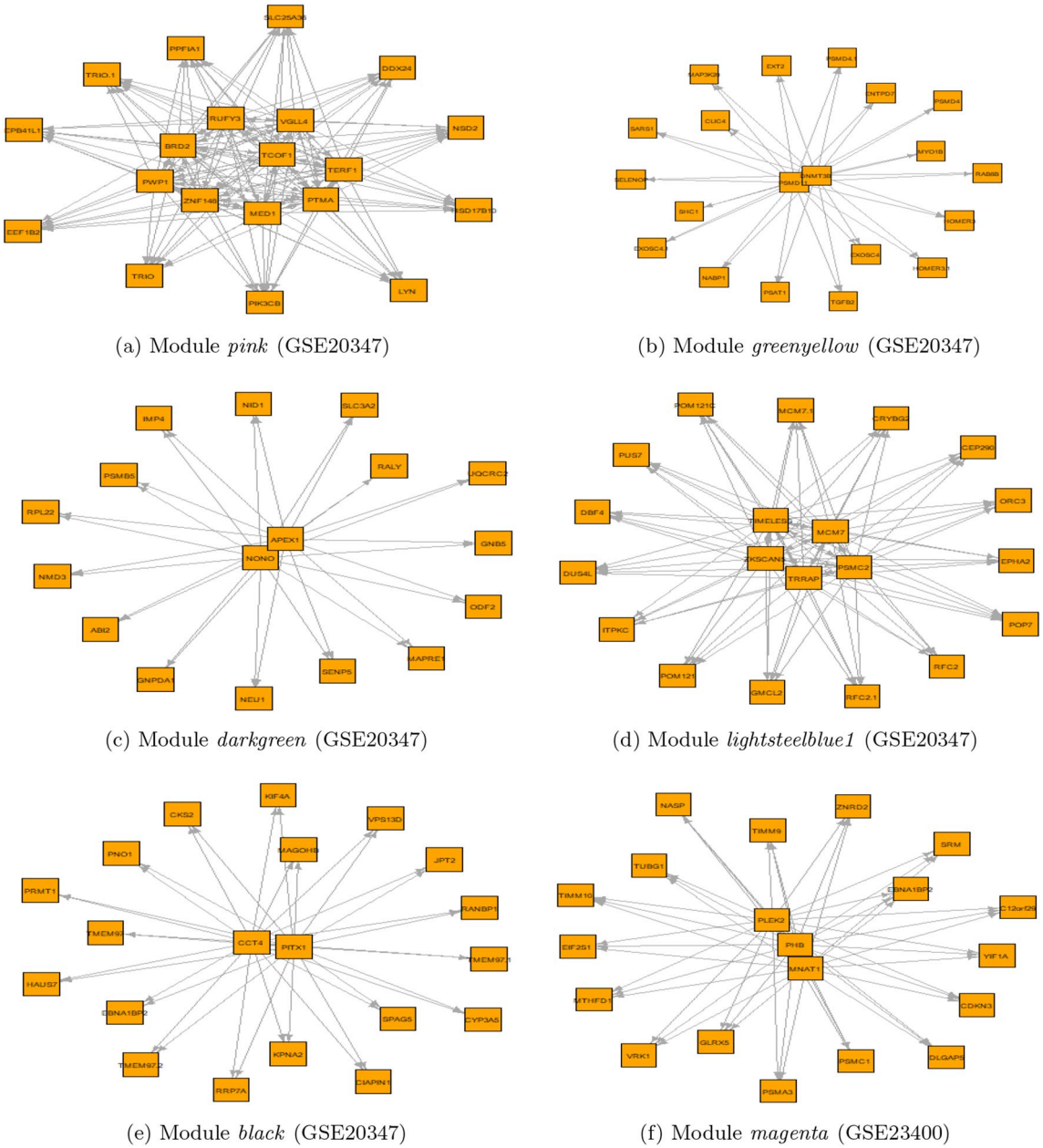
differentiation and lymph node metastasis in ESCC. Ma et al. [29] suggested that Karyopherin α 2 (*KPNA2*) protein levels were high in ESCC tumors, and siRNA against *KPNA2* could inhibit the growth of ESCC cells, suggesting it may be a new potent marker and therapeutic target for ESCC. Sakai et al. [30] further concluded that *KPNA2* expression is associated with poor differentiation, tumor invasiveness, and tumor proliferation in ESCC. Wang et al. [31] identified kinesin family member 4A (*KIF4A*) as a facilitator of proliferation, cell cycle, migration, and invasion of ESCC in vivo and in vitro. Similarly, Sun et al. [32] stated that through the Hippo signaling pathway, *KIF4A* regulates the biological function of ESCC cells thus promoting ESCC cell proliferation and migration. Kita et al. [33] demonstrated that the expression of cyclin-dependent kinase subunit 2 (*CKS2*) in ESCC was elevated relative to levels in normal tissue, and that *CKS2* overexpression is associated with the depth of tumor invasion, lymphatic invasion, clinical stage, distant metastasis and poor prognosis. Zheng et al. [34] found that the expression of cytokine induced apoptosis inhibitor 1 (*CIAPIN1*) was statistically correlated with the degree of differentiation, depth of invasion, and lymph node metastasis of ESCC and thus has been considered as a valuable prognostic indicator in ESCC. Zhao et al. [35] highlighted that Protein arginine methyltransferase 1 (*PRMT1*) activates and maintains esophageal TICs by mediating transcription alteration through histone H4 arginine methylation. Zhou et al. [36] highlighted that *PRMT1* activates Hedgehog signaling and up-regulated the expression of target genes downstream of Hedgehog signaling thus taking an oncogenic role of *PRMT1* in the progression of ESCC.

Zhang et al. [37] provided evidence that Human Leukocyte Antigen-F (*HLA-F*) antigen expression was associated with survival in patients with ESCC. Yie et al. [38] established that Human Leukocyte Antigen-G (*HLA-G*) expression has a strong and independent prognostic value in human ESCC. According to Sato et al. [39], high chemokine (CXC motif) ligand 10 (*CXCL10*) expression is an independent prognostic factor and has the potential to serve as a clinically useful marker of the need for adjuvant chemotherapy after surgery in patients with advanced thoracic ESCC. Yuan et al. [40] suggested the tumor promotion role of Interferon-stimulated gene 15 (*ISG15*) in ESCC via *c-MET/*

Table 6 DEGs that are annotated to most enriched GO term in all three GO databases (BP, CC and MF) as well as the most enriched pathway

Dataset	Top enriched DEGs
GSE20347	*TXNRD1, APPL1, FADD, FAS, MAPK1, PIK3R1, STAT1, RAF1, RARA, MAP2K1, PIK3CD, RAC2, MAPK10, PRKACB, AR, PIK3CB, BCR, KRAS, GSK3B, NFKB2, PIK3R2, FLT3LG*
GSE23400	*RAF1, PIK3R1, APPL1, MAP2K1, AR, PRKCB, PRKACB, STAT1, HIF1A, TXNRD1, FADD, RARA, PIK3CD, IL15, RAC2, GSK3B, STAT2, BCR*
GSE130078	*TYMP, PDE4A, PIK3CD, PIP5K1A, GPI, PDE1B, PDE3B, PDE9A, HPGDS, PDE3A, PI4KA*

DEGs with strong literature evidence of association to Esophageal squamous cell carcinoma (ESCC) are marked in Red while hub genes with evidence of association to five other SCC namely, Oral, Tongue, Head and Neck, Tongue or Laryngeal Squamous Cell Carcinoma are marked in Blue



(a) Module *pink* (GSE20347)

(b) Module *greenyellow* (GSE20347)

(c) Module *darkgreen* (GSE20347)

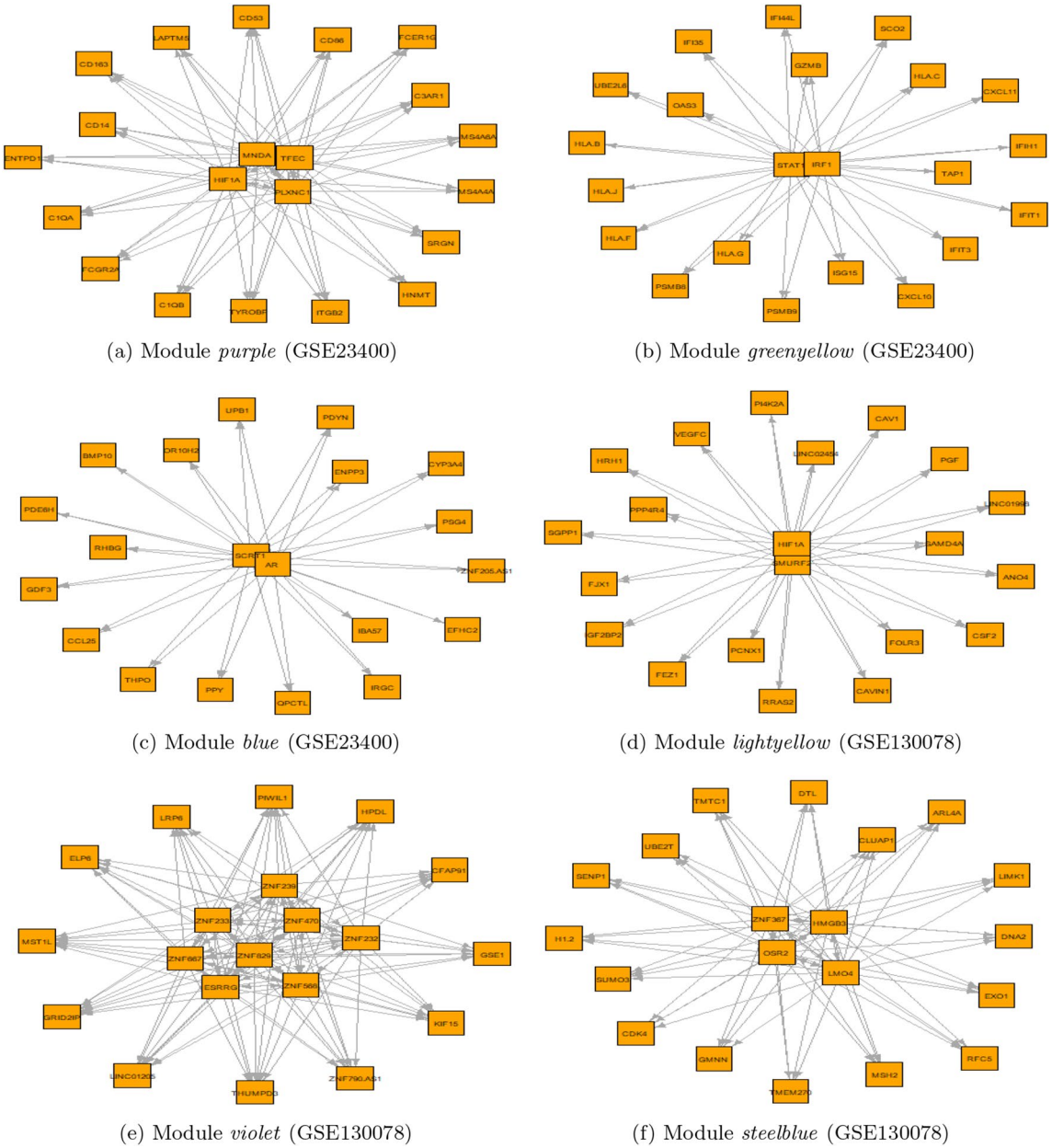
(d) Module *lightsteelblue1* (GSE20347)

(e) Module *black* (GSE20347)

(f) Module *magenta* (GSE23400)

Fig. 5 Gene Regulatory Network (GRN) on the subset of hub genes detected by our method for modules *pink*, *greenyellow*, *darkgreen*, *lightsteelblue1*, *black* and *magenta*. Regulatory behavior is represented by a weighted directed edge from a Transcription Factor (TF) to a Target Gene (TG)

Fyn/α-catenin pathway. Yu et al. [41] and Wang et al. [42] identified that Cyclin-dependent kinase inhibitor 3 (*CDKN3*) regulates tumor progression through activation of AKT signaling pathway in ESCC. Liu et al. [43] further suggested that *CDKN3* acted as an oncogene in human ESCC and may accelerate the *G1/S* transition by affecting CyclinD-CDK4 complex via regulating *pAKT-p53-p21* axis and p27 independent of AKT. Preliminary studies by Hu et al. [44] suggested that disks large-associated protein 5 (*DLGAP5*) promotes cell proliferation in ESCC. According to Liu et al. [45], vaccinia-related kinase (VRK) serine/threonine kinase 1 promotes CDDP resistance through *c-MYC* by activating *c-Jun* and potentiating a malignant phenotype in ESCC. Liu et al. [46] provided a potential target for the immunooncology effect of roteasome alpha-subunit 3 (*PSMA3*) in ESCC therapy. Wang et al. [47] detected the major role of Pleckstrin-2 (*PLEK2*) in driving metastasis and chemoresistance in ESCC by regulating LCN2. Qu et al. [48] found that Component 3a Receptor 1 (*C3AR1*) might be the cause of an immunosuppressive microenvironment by affecting the



(a) Module *purple* (GSE23400)

(b) Module *greenyellow* (GSE23400)

(c) Module *blue* (GSE23400)

(d) Module *lightyellow* (GSE130078)

(e) Module *violet* (GSE130078)

(f) Module *steelblue* (GSE130078)

Fig. 6 Gene Regulatory Network (GRN) on the subset of hub genes detected by our method for modules *purple*, *greenyellow*, *blue*, *lightyellow*, *violet* and *steelblue*. Regulatory behavior is represented by a weighted directed edge from a Transcription Factor (TF) to a Target Gene (TG)

polarization of macrophages to M2 phenotype and lead to the progression of ESCC. Zhang et al. [49] suggested that Signal Transducer and Activator of Transcription-1 (*STAT1*) is a tumor suppressor in ESCC. According to Shao et al. [50], Hypoxia-inducible factor 1α (*HIF*-1α), p53, and vascular endothelial growth factor (VEGF) are important factors that facilitate tumor progression. The results from the study conducted by Hu et al. [51] indicated that *HIF-1α* promotes metastasis of ESCC by targeting SP1 in a hypoxic microenvironment. Bolidong et al. [52] suggested that via *cyclin D1/CDK4-mediated cell cycle* progression Glycogen synthase kinase 3β (*GSK3β*) has a tumor promoting role in ESCC. According to Gao et al. [53], *GSK3β* expression promotes ESCC progression through STAT3 in vitro and in vivo, and GSK3β-STAT3 signaling could be a potential therapeutic target for ESCC treatment.

According to Kato et al. [54] and [55] caveolin-1 (*CAV1*) is a biomarker for ESCC. Lu et al. [56] and Shu et al. [57] indicated that insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*) serves a major carcinogenic role in ESCC. According to [58], the expression of vascular endothelial growth factor C (*VEGF-C*) correlates with

Table 7 Summary of candidate genes detected by our method in the microarray dataset, GS20347 that are annotated to top 3 GO terms in the three GO databases

	GO Term	Annotated Candidate Genes
GO_BP	GO:0007165 signal transduction	*PRKACB, BCR, AR, LYN, APPL1, STAT1, SHC1, NFKB2, PIK3CD, PIK3CB, PIK3R2, PIK3R1, EXT2, RAC2, PPFIA1, RAF1, GNB5, GSK3B, MAPK10, KRAS, RARA, MAP2K1, TXNRD1, FLT3LG, FADD, RANBP1, FAS, MAPK1*
	GO:0045944 positive regulation of transcription from RNA polymerase II promoter	*AR, HOXC11, STAT1, PITX1, NFKB2, TP63, PIK3R2, PIK3R1, PTMA, RAF1, MED1, ZNF148, RARA, FADD, CXCR3*
	GO:0016032 viral process	*ITSN2, POM121, POM121C, LYN, BRD2, PSMB5, STAT1, ABI2, SHC1, PIK3R1, FADD, RANBP1, MAPK1*
GO_CC	GO:0005829 cytosol	*ITSN2, PRKACB, BCR, CASP10, MSRA, RAC2, PTMA, RAF1, UBA7, HOMER3, RARA, MAP2K1, MAP3K20, MCL1, MCM7, UBASH3A, AR, LYN, APPL1, STAT1, KPNA2, PIK3CD, PIK3CB, PIK3R2, PIK3R1, TRIO, RPL22, NEDD4L, PNO1, ABI2, NEB, SARS1, TXNRD1, FADD, CLIC4, PRMT1, FAS, SENP5, EPB41L1, HOXC11, SHC1, ODF2, CEP290, EEF1B2, NABP1, GNB5, KRAS, HAUS7, PSAT1, FLT3LG, MAPK1, PSMD4, RUFY3, PSMB5, TCOF1, PSMC2, NFKB2, JPT2, EXOSC4, CCT4, PPFIA1, SBF1, KIF4A, GSK3B, MAPK10, HPRT1, ITPKC, ENOX2, SPAG5, MAPRE1, RANBP1*
	GO:0005654 nucleoplasm	*PRKACB, CIAPIN1, TP63, MSRA, PTMA, UBA7, SLC3A2, RRP7A, RARA, ORC3, MCL1, MCM7, TIMELESS, NONO, UBASH3A, AR, STAT1, KPNA2, PIK3CB, DBF4, MED1, NEDD4L, PNO1, ABI2, DNMT3B, TXNRD1, PRMT1, SENP5, POP7, MAGOHB, HOXC11, NABP1, TERF1, NSD2, RFC2, NPIPB3, RAB8B, MAPK1, PSMD4, UQCRC2, PSMB5, TCOF1, PSMC2, NFKB2, IMP4, EXOSC4, CCT4, KIF4A, GSK3B, MAPK10, TRRAP, POM121, ZNF148, BRD2, ESF1, NMD3*
	GO:0016020 membrane	*TFRC, BCR, ITGB7, CEP290, NEU1, EXT2, RAC2, GNAQ, SLC3A2, MAN1C1, KRAS, MCL1, MCM7, FLT3LG, NONO, LSS, RUFY3, APPL1, PSMC2, KPNA2, PIK3CD, PIK3CB, ENTPD7, PIK3R1, CD52, CD48, SBF1, KIF4A, DDX24, MED1, LST1, NMD3, CLIC4, FAS*
GO_MF	GO:0005515 protein binding	*TFRC, PRKACB, BCR, ZKSCAN5, NEU1, MSRA, RAC2, RAF1, RALY, CD2, HOMER3, RRP7A, APOOL, RARA, MAP2K1, MCL1, MCM7, TIMELESS, UBASH3A, AR, APPL1, STAT1, PITX1, PIK3CD, PIK3CB, HSD17B10, EPHA2, PIK3R2, PIK3R1, RCN1, MED1, RPL22, NEDD4L, PNO1, ABI2, DNMT3B, TXNRD1, GMCL2, FADD, ITM2A, CLIC4, PRMT1, FAS, TGFB2, POP7, EPB41L1, MAGOHB, HOXC11, CNPY2, SHC1, CEP290, MAN1C1, KRAS, TERF1, RFC2, PSAT1, FLT3LG, RAB8B, VGLL4, MAPK1, UQCRC2, CKS2, TCOF1, NFKB2, IMP4, EXOSC4, KIF4A, GSK3B, MAPK10, TNS1, RANBP1*
	GO:0042802 identical protein binding	*TFRC, PSMD4, APPL1, STAT1, CEP290, TP63, CD3D, RAF1, RALY, CD2, KRAS, HOMER3, RPL22, HPRT1, TERF1, ABI2, PSAT1, FADD, PRMT1, MAPRE1, FAS, TIMELESS, NONO, MAPK1*
	GO:0003723 RNA binding	*POP7, TFRC, PSMD4, MAGOHB, TCOF1, KPNA2, HSD17B10, CCT4, NABP1, DDX24, SLC3A2, RALY, RPL22, RRP7A, PNO1, EBNA1BP2, MAP3K20, SARS1, ENOX2, ESF1, TNS1, NMD3, PRMT1, MAPRE1, PUS7, NONO*

lymph node metastasis and poor prognosis. Similarly, [59] suggests that *VEGF-C* expression in ESCC may play a great key role in lymphatic spread. Feng et al. [60] indicated that ras-like without CAAX1 (*RIT1*) displays tumor-suppressing functions in ESCC, and these functions were carried out by inhibiting *MAPK* and *PI3K/AKT signaling* pathway, inhibiting EMT, and downregulating cancer stemness of ESCC cells. Zhou et al. [61] demonstrated that Dehydrogenase/reductase member 2 (*DHRS2*) had an important part in ESCC development and progression. Li et al. [62] suggested a tumor suppression function for RNA Binding Motif Single Stranded Interacting Protein 3 (*RBMS3*) gene in ESCC. According to Wang et al. [63], *UBE2T* is involved in the development of ESCC, and gene signatures derived from UBE2T-associated genes are predictive of prognosis in ESCC. Gao et al. [64] demonstrated that High Mobility Group Box 3 (*HMBG3*) may be a potential molecular marker for predicting the prognosis of ESCC patients. According to Huang et al. [65], cyclin-dependent kinase 4 (*CDK4*) amplification was identified as an independent prognostic factor for survival, which could be incorporated into the tumor-node-metastasis staging system to refine risk stratification of patients with esophageal squamous cell carcinoma. Ling et al. [66] suggested that MutS Homolog 2 (*MSH2*) methylation in the plasma would be a good predictor of DFS for these ESCC patients before oesophagectomy. Xu et al. [67] identified Estrogen-related receptor gamma (*ESRRG*) as one of four molecular markers that may be helpful

Table 8 Summary of candidate genes detected by our method in the microarray dataset GSE23400 that have been annotated to top 3 GO terms in the three GO databases

Data base	GO Term	Annotated Candidate Genes
GO_BP	GO:0007165 signal transduction	*BCR, AR, APPL1, STAT1, STAT2, CXCL10, CXCL11, PIK3CD, PIK3R1, RAC2, CD53, IL15, TYROBP, RAF1, GSK3B, PRKCB, HIF1A, RARA, MAP2K1, VRK1, TXNRD1, FADD*
	GO:0045944 positive regulation of transcription from RNA polymerase II promoter	*AR, STAT1, STAT2, CXCL10, PIK3R1, RAF1, HIF1A, RARA, FADD, TFEC*
	GO:0045893 positive regulation of transcription	*PRKCB, AR, HIF1A, RARA, STAT1, MAP2K1, CD86*
GO_CC	GO:0005829 cytosol	*BCR, MTHFD1, SCO2. SRM, GZMB, RAC2, RAF1, PRKCB, IFIH1, RARA, MAP2K1, IFIT1, IFIT3, OAS3, AR, APPL1, STAT1, STAT2, HNMT, PIK3CD, UBE2L6, PIK3R1, LAPTM5, CD163, TXNRD1, MNDA, FADD, DLGAP5, CDKN3, IL15, VRK1, EIF2S1, PSMA3, PSMB8, PSMB9, PSMC1, IFI35, ISG15, GSK3B, HIF1A, TUBG1*
	GO:0005654 nucleoplasm	*MNAT1, PRKCB, RARA, OAS3, AR, STAT1, STAT2, HNMT, UBE2L6, TXNRD1, MNDA, IL15, NASP, VRK1, PSMA3, PSMB8, PSMB9, PSMC1, ISG15, GSK3B, HIF1A, TFEC*
	GO:0016020 membrane	*BCR, MTHFD1, ITGB2, GZMB, RAC2, PLXNC1, HLA-C, HLA-F, HLA-G, EIF2S1, OAS3, APPL1, PSMC1, PIK3CD, IFI35, ENTPD1, PIK3R1, TAP1, CD163*
GO_MF	GO:0005515 protein binding	*BCR, SCO2, MNAT1, SRM, RAC2, FCER1G, RAF1, PRKCB, RARA, MAP2K1, AR, APPL1, STAT1, STAT2, PIK3CD, UBE2L6, ENTPD1, PIK3R1, GLRX5, CD163, TXNRD1, FADD, IL15, TIMM9, PLXNC1, VRK1, EIF2S1, FCGR2A, IFI35, GSK3B, HIF1A, TUBG1*
	GO:0042802 identical protein binding	*APPL1, STAT1, STAT2, SRM, IFI35, CD53, FCER1G, TYROBP, RAF1, IFIH1, IFIT3, HLA-G, FADD, TUBG1, C1QB*
	GO:0003723 RNA binding	*PSMC1, IFIH1, EBNA1BP2, IFIT1, IFIT3, EIF2S1*

in the diagnosis and treatment of ESCC. Chen et al. [68] demonstrated that silencing *EphA3* in *KYSE410 cells* triggers epithelial–mesenchymal transition, and promoted cell migration and invasion in ESCC. Luo et al. [69] found that knockdown of Insulin-like growth factor binding protein-3 (*IGFBP-3*) confers resistance to the cell killing effects of IR on ESCC in vitro and in vivo. Zhao et al. [70] indicated that the increased ESCC chemosensitivity might be dependent on *IGFBP-3* upregulation through *EGFR-dependent* pathway. Furthermore, according to Luo et al. [71], high level of *IGFBP-3* expression in ESCC associates with early clinical stages and are predictive for favorable survival of the patients treated with radiotherapy. According to Wang et al. [72], carbohydrate sulfotransferase 15 (*CHST15*) promotes the proliferation of TE-1 cells via multiple pathways in ESCC.

Discussion

In Table 11, we give a detailed summary of all DEGs that have been identified by our method as candidates for potential biomarkers for ESCC. In our method, we consider strong literature evidence for association with ESCC and five other SCCs related to ESCC as the necessary criterion for a candidate gene to be a potential biomarker, and the findings from literature are summarized in Table 11. In the table, we also highlight the enriched GO terms and pathways to which the candidate genes has been annotated. Furthermore, it also details whether the same is a hub gene, a transcription factor (TF) or whether it is upregulated or down-regulated. A DEG is upregulated if $logFC > 0$ and downregulated when $logFC < 0$. We take into consideration $logFC$ values calculated by limma for the microarray datasets, and edgeR in the RNA-Seq dataset.

The biological relevance of a candidate to its respective dataset is considered based on three criteria:

(a) Annotated to at least one GO term in 2 of 3 GO databases with p value ≤ 0.05,
(b) Annotated to at least one KEGG pathway with p value ≤ 0.05, and
(c) It's a TF and thus exhibits regulatory behavior towards other DEGs in the network.

For a candidate gene to be considered a potential biomarker, we consider following four cases.

Case 1: Strong literature evidence of association with ESCC and biologically relevant to its dataset based on all three criteria a,b and c,

Table 9 Summary of candidate genes detected by our method in GS130078 that have been annotated to top 3 GO terms in the three GO databases

Data base	GO Term	Hub Genes
GO_BP	GO:0007165 signal transduction	*PIP5K1A, RHBDL1, RRAS2, CXCL16, HPGDS, PIK3CD, PIK3CB, VEGFC, RIT1, RALB, CDC42SE1, HIF1A, GPI, PGF, CDK4, PDE1B, PDE2A, PDE3A, TENM1, TYMP, PDE9A, CSF2RB, PI4KA, PI4KB, PDE3B, PDE4A, PDE4D, PPP2R5A, PDE5A, GNA13, LIMK1*
	GO:0000122 negative regulation of transcription from RNA polymerase II promoter	*MEF2A, H1-2, ZNF239, ZNF85, OSR2, CAV1, NFIB, ZNF568, PDE2A*
	GO:0045944 positive regulation of transcription from RNA polymerase II promoter	*TBK1, MEF2A, LRP6, OSR2, ZNF91, HIF1A, NFIB, ESRRG, LMO4, SENP1*
GO_CC	GO:0005886 plasma membrane	*FEZ1, KCNH3, ARL4A, SDE2, RIT1, RALB, PI4K2A, COA6, TENM1, CDH22, CDH23, RRAS2, CXCL16, PIK3CD, PIK3CB, LRP6, EPHA3, CAVIN1, CARMIL1, PDE2A, PDE9A, PDE4A, PDE4D, PIP5K1A, ADGRL3, EXO1, SYT15, CACNA1B, CACNA1E, CAV1, GPI, CSF2, HRH1, PI4KA, GNA13, ZAN, PCDH10, ANO4, CDC42SE1, CDH4, MUCL1*
	GO:0005829 cytosol	*ARFGAP1, ARL4A, SDE2, PI4K2A, ELP6, TYMP, NEXMIF, SAMD4A, HPGDS, PIK3CD, PIK3CB, DTL, EPHA3, CAVIN1, CHPF, PPP6C, PDE1B,CARMIL1, PDE2A, PDE3A, PDE9A, PDE3B, PDE4A, PDE4D, PDE5A, TBK1, PIP5K1A, CRLF1, GMNN, IGF2BP2, MON2, GPI, POLR2D, PANK2, HRH1, PI4KA, PI4KB, PPP2R5A, RBMS3, GNA13, LIMK1, PPP4R4, MEF2A, THUMPD3, KIF15, CCT2*
	GO:0005654 nucleoplasm	*MSH2, ARL4A, SDE2, ZNF85, ERCC3, NFIB, COA6, NEXMIF, HPGDS, PIK3CB, SUMO3, DTL, EPHA3, CAVIN1, APPBP2, RNF112, ESRRG, PPP6C, RBBP5, CARMIL1, PDE9A, CTDSPL2, PDE4A, SENP1, TBK1, PIP5K1A, LIN52, ZNF470, GMNN, EXO1, UBE2T, GPI, ZNF367, DHRS2, RFC5, POLR2D, DNA2, MEF2A, ZNF232, CLUAP1, HIF1A, CDK4*
GO_MF	GO:0005515 protein binding	*ORAI2, KCNH3, MSH2, PIWIL1, SDE2, RALB, XIRP1,GSE1, TYMP, RRAS2, HPGDS, MFHAS1, PIK3CD, PIK3CB, DTL, LRP6, EPHA3, CRELD2, APPBP2, RNF112, ESRRG, RBBP5, PDE1B, PDE2A, PDE3A, IGFN1, PDE9A, SOHLH1, PDE3B, PDE4A, PDE4D, PDE5A, ACAN, PIP5K1A, LIN52, CRLF1, TMBIM4, VEGFC, CACNA1B, UBE2T, GNS, GPI, RFC5, DNA2, PI4KA, PI4KB, PPP2R5A*
	GO:0042802 identical protein binding	*TBK1, CAV1, PGF, EMILIN3, LRP6, PCSK1, CAVIN1, ESRRG, PDE2A, PDE9A*
	GO:0004712 protein serine/threonine/tyrosine kinase activity	*TBK1, CDK18, CDK4, EPHA3, LIMK1*

Case 2: Strong literature evidence of association with ESCC and biologically relevant to its dataset based on criteria a and b,

Case 3: Strong literature evidence of association with ESCC and biologically relevant to its dataset based on criteria a or b,

Case 4: Biologically relevant to its dataset using all three criteria a,b and c, and has literature evidence of association with previously mentioned 5 SCCs related to ESCC, namely, oral SCC, Lung SCC, Tongue SCC, Head and Neck SCC and Laryngeal SCC.

All candidate genes that fall under Case 1 and Case 2 are considered potential biomarkers for ESCC because of existing evidence of association with ESCC in the form of other works while our biological validation of these genes establishes their relevance to their respective datasets. For candidate genes that fall under Case 3, although there is strong literature evidence of association with ESCC, we have weak evidence of their biological relevance to their datasets. On the other hand, for candidate genes that fall under Case 4, although we strongly validate their biological relevance to their datasets, there is only literature evidence of association with other SCCs related to ESCC. For both these cases, the candidates can be considered probable potential biomarkers, but need further in-depth analysis.

Top Enriched DEGs (TEDs), *STAT1* and *HIF1A* detected in both microarray datasets (GSE20347 and GSE23400) and GSE130078, respectively, belong to Case 1. In GSE20347, two candidates *DNMT3B* and *MCM7* also belong to Case 1.

Table 10 Summary of candidate genes detected by our method in all three databases that have been annotated to the top 5 KEGG enriched pathways in these two microarray datasets

Data set	KEGG Pathways	Annotated Candidate Genes
GSE20347	hsa05200:Pathways in cancer	*BCR, AR, APPL1, CKS2, RARA, STAT1, MAP2K1, NFKB2, PIK3CD, PIK3CB, TXNRD1, FLT3LG, PIK3R2, PIK3R1, FADD, RAC2, FAS, RAF1, TGFB2, GNAQ, GNB5, GSK3B, MAPK1, MAPK10, KRAS*
	hsa04010:MAPK signaling pathway	*PRKACB, FLT3LG, EPHA2, RAC2, FAS, MAP2K1, RAF1, MAP3K20, TGFB2, NFKB2, MAPK1, MAPK10, KRAS*
	hsa05169:Epstein-Barr virus infection	*PSMD4, PIK3R2, PIK3R1, FADD, LYN, CD3D, STAT1, PSMC2, FAS, NFKB2, PIK3CD, PIK3CB, MAPK10*
	hsa04151:PI3K-Akt signaling pathway	*FLT3LG, EPHA2, PIK3R2, PIK3R1, MAP2K1, RAF1, MCL1, GNB5, GSK3B, PIK3CD, PIK3CB, ITGB7, MAPK1, KRAS*
	hsa05171:Coronavirus disease - COVID-19	*RPL22, PIK3R2, PIK3R1, STAT1, PIK3CD, PIK3CB, MAPK1, MAPK10*
GSE23400	hsa05200:Pathways in cancer	*PRKCB, BCR, AR, HIF1A, APPL1, RARA, STAT1, STAT2, MAP2K1, PIK3CD, TXNRD1, PIK3R1, FADD, RAC2, IL15, RAF1, GSK3B*
	hsa04010:MAPK signaling pathway	*PRKCB, CD14, RAC2, MAP2K1, RAF1*
	hsa04151:PI3K-Akt signaling pathway	*PIK3R1, MAP2K1, RAF1, GSK3B, PIK3CD*
	hsa05169:Epstein-Barr virus infection	*ENTPD1, HLA-C, HLA-F, HLA-G, PIK3R1, FADD, STAT1, STAT2, PSMC1, TAP1, ISG15, CXCL10, PIK3CD, OAS3*
	hsa05171:Coronavirus disease - COVID-19	*C3AR1, PRKCB, PIK3R1, IFIH1, C1QB, C1QA, STAT1, STAT2, ISG15, CXCL10, PIK3CD, OAS3, FCGR2A*
GSE130078	hsa01100:Metabolic pathways	*PIP5K1A, PIGT, HPGDS, PIK3CD, PIK3CB, ST6GALNAC5, ST6GAL1, MOGS, ARSB, NAT8B, PI4K2A, GNS, GPI, CHPF, PANK2, PDE1B, PDE2A, PDE3A, TYMP, PDE9A, PI4KA, PI4KB, PDE3B, PDE4A, PDE4D, PDE5A, SGPP1*
	hsa05200:Pathways in cancer	*HIF1A, PGF, MSH2, CDK4, PIK3CD, PIK3CB, LRP6, VEGFC, CSF2RB, RALB, GNA13*
	hsa04144:Endocytosis	*PIP5K1A, CAV1, ARFGAP1, FOLR3*
	hsa04010:MAPK signaling pathway	*RRAS2, VEGFC, PGF, CACNA1B, CACNA1E*
	hsa05165:Human papillomavirus infection	*TBK1, CDK4, PIK3CD, PIK3CB, PPP2R5A*

Thus, *STAT1, HIF1A, DNMT3B* and *MCM7* are potential biomarkers for ESCC. *GSK3B* is a TED detected in both microarrays, and belongs to Case 2. In dataset GSE20347, 9 candidate genes *HOMER3*, *PSMD4*, *PSTAT1*, *TFRC*, *MCL1*, *EPHA2*, *KPNA2*, *CKS2* and *PRMT1* belong to Case 2, and thus are potential biomarkers for ESCC. Similarly, 7 candidate genes *HLA-F*, *HLA-G*, *CXCL10*, *ISG15*, *PSMA3*, *FCGR2A* and *C3AR1* are potential biomarkers for ESCC as they fall under Case 2. Four candidate genes in the RNASeq dataset GSE130078, *CAV1*, *VEGFC*, *CDK4* and *MSH2* fall under Case 2 and are potential biomarkers for ESCC.

Three candidates genes in GSE20347, *PTMA*, *VGLL4* and *NONO* fall in Case 3. In other words, although there are other works that establish their role as potential biomarkers for ESCC, the biological relevance to their respective datasets is not that strong. However, they can still be regarded as probable potential biomarkers for ESCC, but need further in-depth validation. Similarly in GSEE23400 and GSE130078, one (*PLEK2*) and 2 (*HMGB3* and *ESRRG*) genes fall under Case 3. *PSMC2* detected in GSE20347, on the other hand falls under Case 4. We validate its strong association with the dataset as this candidate gene has been annotated to GO terms in all three GO databases as well as several enriched pathways. They further exhibit regulatory behavior in a GRN, but there are no previous works that relate the same to ESCC. However, its worth mentioning that there is literature evidence that identify *PSMC2* as potential biomarker for OSCC. Similarly, the TED identified in the two microarray datasets, *AR*, also falls under Case 4. Both, *PSMC2* and *AR* are probable potential biomarkers for ESCC, but need further in-depth analysis.

In Table 12, we put forward a comparison between our work and two recent works presented by Patowary et al. [166] and Hu. et al. [44] that perform DE analysis by employing approaches and methods similar to our work.

Conclusion

All six methods, three microarray and three RNA-sequencing, employed by the proposed integrative approach based differential expression (DE) Analysis framework were found effective in extracting differentially expressed genes (DEGs) with a p-value of 0.05. We further proposed a consensus function that takes into account the information loss due to the DEGs common to all three respective methods and

Table 11 Summary of potential biomarkers identified by our proposed framework (Fig. 1)

	Detected hub gene	Enriched GO Term	Enriched cancer pathway(s)	HG or TED	TF?	+/–	Literature Evidence
GSE20347	*PTMA*	BP, CC, MF	Nil	Yes	Yes (Fig 5a)	+	ESCC [13], HNSCC [73]
	PPFIA1	BP, CC	Nil	HG	No		ESCC [14], HNSCC [74]
	PIK3CB	BP, CC, MF	hsa05200,hsa05169,hsa04151,hsa05171, hsa05165, hsa05417,hsa05161,hsa04510, hsa04015,hsa05162, hsa05164,hsa05205, hsa05166,hsa04210,hsa05418, hsa05167, hsa05163,hsa04380,hsa05146,hsa05222, hsa04933,hsa05135,hsa05215,hsa05017, hsa04722, hsa04722,hsa05170,hsa04810, hsa04066,hsa01100, hsa05131,hsa05010, hsa04024,hsa04014,hsa05210, hsa05221, hsa05160,hsa05212,hsa04218,hsa04630, hsa05203	HG	No		OSCC [75]
	VGLL4	BP, CC, MF	Nil	HG	Yes (Fig. 5a)	+	ESCC [15]
	HOMER3	BP, CC, MF	hsa04068,hsa04724	HG	No	+	ESCC [16]
	SHC1	BP, CC, MF	hsa04510,hsa04722,hsa04014,hsa04072, hsa04935, hsa04910,hsa01521,hsa04062, hsa05220,hsa05225, hsa05226,hsa04917, hsa05224,hsa04915,hsa04012, hsa05214, hsa04926,hsa01522,hsa04650,hsa05100	HG	No	+	LSCC [76]
	PSMD4	BP, CC, MF	hsa05169,hsa05017,hsa05022,hsa05010, hsa05020, hsa05016,hsa05014,hsa05012 ,hsa03050	HG	No	+	ESCC [17]
	CLIC4	BP, CC, MF	Nil	HG	No	+	HNSCC [77]
	DNMT3B	BP, CC, MF	hsa01100	HG	Yes (Fig. 5b)	+	ESCC [78], HNSCC [79], OSCC
	MYO1B	BP, CC, MF	hsa05130	HG	No	+	HNSCC [80]
	PSAT1	CC, MF	hsa01100,hsa01200	HG	No	+	ESCC [19]
	SLC3A2	BP, CC, MF	hsa04150,hsa04216	HG	NO	+	OSCC [81]
	NONO	BP, CC, MF	Nil	HG	Yes (Fig. 5c)		ESCC [20]
	TFRC	BP, CC, MF	hsa04066,hsa04145,hsa04640,hsa04144, hsa04216	HG	No	+	ESCC [21]
	SENP5	BP, CC	Nil	HG	No	+	OSCC [82]
	RCN1	BP, CC, MF	Nil	HG	No	+	OSCC [83]
	CNPY2	BP, CC, MF	Nil	HG	No	+	ESCC [22]
	SBF1	BP, CC, MF	Nil	HG	No	–	HNSCC [84]
	MCL1	BP, CC, MF	hsa04151,hsa04210,hsa04630	HG	No	+	ESCC [23], OSCC [85]
	POP7	BP, CC, MF	Nil	HG	No	+	ESCC [24]
	MCM7	BP, CC, MF	hsa04110,hsa03030	HG	Yes (Fig. 5d)	+	ESCC [25–27], OSCC [86]
	POM121	BP, CC, MF	hsa03013,hsa05014	HG	No		OSCC [87]

Table 11 (continued)

	Detected hub gene	Enriched GO Term	Enriched cancer pathway(s)	HG or TED	TF?	+/–	Literature Evidence
	PSMC2	BP, CC, MF	hsa05169,hsa05017,hsa05022,hsa05010, hsa05020, hsa05016,hsa05014,hsa05012 ,hsa03050	HG	Yes (Fig 5d)	+	OSCC [88]
	EPHA2	BP, CC, MF	hsa04010,hsa04151,hsa04015,hsa04014, hsa04360	HG	No	+	ESCC [28] ,LSCC [89], HNSCC [90, 91]
	KPNA2	BP, CC, MF	hsa05164,hsa03013,hsa05207	HG	No	+	ESCC [29, 30], OSCC [92]
	KIF4A	BP, CC, MF	Nil	HG	No	+	ESCC [31, 32], OSCC [93]
	CCT4	BP, CC, MF	Nil	HG	Yes (Fig. 5e)	+	HNSCC [94]
GSE20347	*CKS2*	BP, CC, MF	hsa05200,hsa05222	HG	No	+	ESCC [33], TSCC [95]
	CIAPIN1	BP, CC, MF	Nil	HG	No	+	ESCC [34]
	PRMT1	BP, CC, MF	hsa04068,hsa04922	HG	No	+	ESCC [35, 36], HNSCC [96]
	HPRT1	BP, CC, MF	hsa01100	HG	No	+	OSCC [97], HNSCC [98]
	NDD4L	Nil	Nil	HG	No	+	OSCC [99]
	KRAS	BP, CC, MF	hsa05200,hsa04010,hsa04151,hsa05165, hsa05417, hsa05161,hsa04015,hsa05205, hsa05166,hsa04210, hsa05167,hsa05163, hsa04933,hsa05215,hsa05022, hsa04722, hsa05170,hsa04810,hsa04066,hsa05010, hsa04014,hsa05210,hsa05221,hsa05160, hsa05212, hsa04218,hsa05203,hsa04072, hsa04919,hsa04935, hsa04660,hsa04068, hsa04910,hsa01521,hsa04062, hsa05211, hsa05220,hsa05225,hsa04625,hsa05226, hsa05223,hsa05230,hsa04917,hsa05224, hsa04540, hsa04915,hsa04550,hsa05218, hsa04012,hsa05214, hsa04071,hsa04720, hsa04926,hsa05213,hsa05219, hsa05208 ,hsa05235,hsa04140,hsa04662,hsa04912, hsa05231,hsa01522,hsa04650,hsa04929, hsa04664, hsa04360, hsa04730,hsa04916 ,hsa04371,hsa04921, hsa05216,hsa04725 ,hsa04213,hsa04211,hsa04137, hsa04960 ,hsa04370,hsa04150,hsa05207,hsa04726	TED	No	–	LSCC [100]
	PIK3R2	BP, CC, MF	Nil	TED	No	–	LSCC [101]
GSE23400	*TAP1*	BP, CC, MF	hsa05169,hsa05163,hsa05170,hsa04145, hsa04612	HG	No	+	TSCC [102]
	HLA-F	BP, CC, MF	hsa05169,hsa05165,hsa05166,hsa05163 ,hsa05167, hsa05170,hsa05203,hsa04 218,hsa04145,hsa04144, hsa04612,hs a05416,hsa05320,hsa04940,hsa05332, hsa04514,hsa05330	HG	No	+	ESCC [37]

Table 11 (continued)

	Detected hub gene	Enriched GO Term	Enriched cancer pathway(s)	HG or TED	TF?	+/–	Literature Evidence
	IFIT3	BP, CC, MF	Nil	HG	No	+	OSCC [103, 104]
	HLA-G	BP, CC, MF	hsa05163,hsa05167,hsa05170,hsa05203, hsa04218, hsa04145,hsa04144,hsa04612, hsa05416,hsa05320, hsa04940,hsa05332, hsa04514,hsa05330	HG	No	+	ESCC[38] , HNSCC [105, 106], OSCC [107]
	IFI44L	BP, CC, MF	Nil	HG	No	+	OSCC [108]
	CXCL10	BP, CC, MF	hsa05169,hsa05171,hsa05164,hsa05160, hsa04060, hsa04062,hsa04668,hsa04620, hsa04622,hsa04657, hsa04061,hsa04623	HG	No	+	ESCC [39], TSCC [109], HNSCC [110]
	IFIT1	BP, CC, MF	hsa05160	HG	No	+	OSCC [103], HNSCC [111]
	ISG15	BP, CC, MF	hsa05169,hsa05171,hsa05165, hsa04622	HG	No	+	ESCC [40] , OSCC [112, 113]
	CDKN3	BP, CC, MF	Nil	HG	No	+	ESCC [41–43]
	DLGAP5	BP, CC, MF	Nil	HG	No	+	ESCC [44]
	VRK1	BP, CC, MF	Nil	HG	No	+	ESCC [45], HNSCC [114]
	PSMA3	BP, CC, MF	hsa05017,hsa05022,hsa05010,hsa05020, hsa05016, hsa05012,hsa05014,hsa03050	HG	No	+	ESCC [46], OSCC [115]
	PLEK2	BP, CC	Nil	HG	Yes (Fig. 5f)	+	ESCC [47], HNSCC [116]
	CD163	BP, CC, MF	Nil	HG	No	+	HNSCC [117], OSCC [118, 119]
	ITGB2	BP, CC, MF	hsa04015,hsa05166,hsa05152,hsa05146 ,hsa04810, hsa04145,hsa04610,hsa05 134,hsa05140,hsa04613, hsa05416,hs a04650,hsa04670,hsa04390,hsa05133, hsa05323,hsa05144,hsa04514	HG	No	+	OSCC [120]
	FCGR2A	BP, CC, MF	hsa05171,hsa05152,hsa04380,hsa05135, hsa04145, hsa05130,hsa04611,hsa04666, hsa05140,hsa04613	HG	No	+	ESCC [121] , HNSCC [122, 123]
	C3AR1	BP, CC, MF	hsa05171,hsa04936,hsa04080,hsa04610	HG	No	+	ESCC [48]
	HIF1A	BP, CC, MF	hsa05200,hsa05205	TED	Yes 6a	+	ESCC[50, 51], OSCC [124–126], TSCC [127]
	CAV1	BP, CC, MF	hsa04144,hsa04510,hsa05205	HG	No	+	ESCC[54, 55], OSCC[128], HNSCC[129], TSCC[130]
GSE130078	*IGF2BP2*	BP, CC, MF	Nil	HG	No	+	ESCC[56, 57], OSCC[131, 132]
	VEGFC	BP, CC, MF	hsa05200,hsa04010,hsa04151,hsa04015, hsa04510, hsa04020,hsa04926	HG	No	+	ESCC[58, 59], OSCC[133, 134]
	HIF1A	BP, CC, MF	hsa05200,hsa05205	HG	Yes 6d	+	ESCC[50, 51], OSCC [124–126], TSCC [127]
	GNA13	BP, CC, MF	hsa05200,hsa04270	HG	No	+	LSCC [135]
	RIT1	BP, CC	Nil	HG	No	-	ESCC [60]
	DHRS2	BP, CC	Nil	HG	No	+	ESCC [61]

Table 11 (continued)

	Detected hub gene	Enriched GO Term	Enriched cancer pathway(s)	HG or TED	TF?	+/–	Literature Evidence
	RBMS3	BP, CC, MF	Nil	HG	No	+	ESCC [62], LSCC [136]
	RFC5	BP, CC, MF	Nil	HG	No	+	LSCC [137]
	UBE2T	BP, CC, MF	Nil	HG	No	+	ESCC [63]
	HMGB3	BP, CC, MF	Nil	HG	Yes 6f	+	ESCC [64]
	LMO4	BP, CC, MF	Nil	HG	Yes 6f	+	HNSCC [138], TSCC[139]
	CDK4	BP, CC, MF	hsa05200,hsa05165,hsa04151,hsa04530, hsa05224, hsa04110	HG	No	+	ESCC [65], HNSCC[140, 141]
	EXO1	BP, C C, MF	Nil	HG	No	+	HNSCC[142]
	MSH2	BP, CC, MF	hsa05200	HG	No	+	ESCC[66], HNSCC [143]
	TBK1	BP, CC, MF	hsa05165	HG	No	+	HNSCC [144]
	HPDL	CC, MF	Nil	HG	No	+	TSCC [145]
	LRP6	BP, CC, MF	hsa05200,hsa05224	HG	No	+	OSCC [146]
	ESRRG	BP, CC, MF	Nil	HG	Yes 6e	–	ESCC [67], LaSCC [147]
	EPHA3	BP, CC, MF	Nil	HG	Yes	+	ESCC [68]
	TRAM2	CC	Nil	HG	Yes	+	OSCC [148]
	IGFBP3	BP, CC, MF	Nil	HG	Yes	+	ESCC [69–71], OSCC[149, 150]
	CLPTM1L	BP, CC	Nil	HG	No	+	OSCC[151, 152]
	CHST15	CC	Nil	HG	No	+	ESCC[72]
GSE20347 & GSE23400	*TXNRD1*	BP, CC, MF	hsa05200,hsa05225	TED	No	+	OSCC [153], HNSCC [154]
	FADD	BP, CC, MF	hsa05200,hsa05169,hsa05165,hsa05161, hsa05162, hsa05164,hsa04210,hsa05167, hsa05152,hsa05163, hsa05022,hsa05170, hsa04936,hsa05010,hsa05160, hsa05132, hsa05142,hsa04668,hsa04620,hsa05130, hsa01524,hsa04622,hsa04657,hsa04217 ,hsa04621	TED	No	+	OSCC [155], HNSCC [156, 157]
	STAT1	BP, CC, MF	hsa05200,hsa05169,hsa05171,hsa05165, hsa05161, hsa05162,hsa05164,hsa05167, hsa05152,hsa04380, hsa04933,hsa05145, hsa05160,hsa05212,hsa04630, hsa04919, hsa04935,hsa04062,hsa04625,hsa04620, hsa04659,hsa04917,hsa04658,hsa05140, hsa05235, hsa04217,hsa05321,hsa04621	TED	Yes 6b	+	ESCC [49], HNSCC [158, 159]
	AR	BP, CC, MF	hsa05200,hsa05215,hsa05207	TED	Yes 6c	-	OSCC [160, 161]

Table 11 (continued)

	Detected hub gene	Enriched GO Term	Enriched cancer pathway(s)	HG or TED	TF?	+/–	Literature Evidence
	RAF1	BP, CC, MF	hsa05200,hsa04010,hsa04151,hsa05165, hsa05161, hsa04510,hsa04015,hsa0516 4,hsa05205,hsa04210, hsa05167,hsa05 152,hsa05163,hsa05215,hsa05022, hsa 04722,hsa05170,hsa04810,hsa05010, hsa04024, hsa04014,hsa05210,hsa05221, hsa05160,hsa05212, hsa04218,hsa04630, hsa04072,hsa04919,hsa05132, hsa04935, hsa04660,hsa04068,hsa04910,hsa01521, hsa04062,hsa04022,hsa05211,hsa04928, hsa05220, hsa05225,hsa04625,hsa05226, hsa05223,hsa05230, hsa04917,hsa05224, hsa04540,hsa04915,hsa04550, hsa05218, hsa04012,hsa05214,hsa04071,hsa04720, hsa04926,hsa05213,hsa04666,hsa05219, hsa05208, hsa05235,hsa04140,hsa04662, hsa04270,hsa04912, hsa05231,hsa01522, hsa04650,hsa04613,hsa04929, hsa04664 ,hsa04360,hsa04730,hsa04916,hsa04371, hsa04921,hsa04370,hsa04150,hsa05207 ,hsa04726	TED	No	–	OSCC [162]
GSE20347 & GSE23400	*GSK3B*	BP, CC, MF	hsa05200,hsa04151,hsa05165,hsa05417, hsa04510, hsa05162,hsa05167,hsa05163, hsa05135,hsa05215, hsa05022,hsa04722, hsa05131,hsa05010,hsa05210, hsa05160, hsa04919,hsa04935,hsa04660,hsa04910, hsa01521,hsa04062,hsa05225,hsa05226, hsa04110, hsa04917,hsa05224,hsa04932, hsa04550,hsa04012, hsa04728,hsa04934, hsa05020,hsa05415,hsa05213, hsa04931, hsa04662,hsa04657,hsa04360,hsa04916, hsa04390,hsa04310,hsa04150	TED	No	+	ESCC [52, 53], OSCC [163, 164]

Table 11 (continued)

	Detected hub gene	Enriched GO Term	Enriched cancer pathway(s)	HG or TED	TF?	+/–	Literature Evidence
	MAP2K1	BP, CC, MF	hsa05200,hsa04010,hsa04151,hsa05165, hsa05161, hsa04510,hsa04015,hsa05164, hsa05205,hsa05166, hsa04210,hsa05167, hsa05163,hsa04380, hsa05135,hsa05215, hsa05022,hsa04722,hsa05170, hsa04810, hsa04066,hsa05010,hsa04024,hsa04014, hsa05210,hsa05221,hsa05160,hsa05212, hsa04218, hsa04072,hsa04919,hsa05132, hsa04935,hsa04660, hsa04068,hsa04910, hsa01521,hsa04062,hsa04022, hsa05211 ,hsa04928,hsa05220,hsa04668,hsa05225, hsa04620, hsa05226,hsa05223,hsa05230, hsa04917, hsa05224,hsa04540,hsa04915, hsa04550,hsa05218, hsa04012,hsa04934, hsa05214,hsa04071,hsa04720, hsa04926, hsa05213,hsa04666,hsa05219,hsa05208, hsa05235,hsa04140,hsa04662,hsa04270, hsa04912, hsa05231,hsa01522,hsa04650, hsa04613,hsa04929, hsa04664,hsa04360, hsa04730,hsa04916,hsa04371, hsa04921, hsa04370,hsa05216,hsa04725,hsa04150, hsa05207,hsa04726	TED	No	–	HNSCC [165]

HG hub gene, *TED* Top Enriched DEG, DEG annotated to most enriched GO term in all three databases as well as to the most enriched pathway, *TF* transcription factor, + Upregulated DEG and – Downregulated DEG

Table 12 Comparison of our method with two recent works that employ DE analysis on ESCC datasets

Parameter	Our Method	Patowary et al. [166], 2020	Hu. et al. [44], 2020
Datasets	Microarray: GSE20347 and GSE23400, RNA-Seq: GSE130078	Microarray: GSE20347 and GSE23400, RNA-Seq: SRP064894	Microarray: GSE20347 and GSE26886
Identification of DEGs	DEA methods: Limma, SAM, EBAM, limma-voomm, edgeR and DESeq2 with $p \leq 0.05$	DEA methods: Limma, SAM, EBAM, limma-voomm, edgeR and DESeq2 with $p \leq 0.01$	Limma; $\|log2foldchange(FC)\|) > 2$ and $adjustedP < 0.05$
Consensus function	(a) Common genes given by the methods with $p \leq 0.05$ (b) For each method, DEGs not in common genes with $q \leq 0.05$(RNA-Seq) or $localfdr \leq 0.05$ (Microarray)	(a) Common genes given by the methods with $p \leq 0.01$ and (b) top ranked DEGs (other than common) given by each method with $p \leq 0.001$	Up- and downregulated DEGs common to both microarray datasets
Module Extraction	Heiarchical Tree Classification; Eigenmodule selection and MEDissThres threshold merging	Hierarchical Tree Classification; Eigenmodule selection and MEDissThres threshold merging	(a) PPI network; Nodes with edge of > 20 as hub genes; (b) WGCNA and Heiarchical Tree Classification
Preservation Analysis	Yes	Yes	No
Modules of Interest	All non-preserved modules of size larger that 100 nodes	Least Preserved module	Relevance between each module and hub genes identified in PPI network < 0.5
hub gene	Intramodular Connectivity	Intramodular Connectivity; Degree and confidence in PPI Network	Nodes with edge of > 20 in PPI network
Candidate Identification	Top 20 hub genes in all modules of interest; DEGs that are annotated to most enriched GO term in all three GO databases as well as the most enriched pathway	DEG with highest intramodular connectivity in each module of interest	Top hub gene in module of interest
Enrichment Analyses	DAVID; GO and KEGG pathway	DAVID; GO and pathway	DAVID; GO and pathway and GSEA
Diseases considered for literature trace	ESCC and five other SCCs, namely oral SCC, Lung SCC, Tongue SCC, Head and Neck SCC, Laryngeal SCC	ESCC and all other cancers	ESCC and all other cancers
Identified potential biomarkers	*STAT1,HIF1A,DNMT3B, MCM7,GSK3B,HOMER3, PSMD4,PSTAT1,TFRC, MCL1,EPHA2,KPNA2, CKS2,PRMT1,HLA-F, HLA-G,CXCL10,ISG15, PSMA3,FCGR2A,C3AR1, CAV1,VEGFC,CDK4 and MSH2*	*COL27A1,SOX11,BAG6, TOP3,CDC6,EZH2, COL7A1,G6PD and AKR1C2*	*DLGAP5*

further employs *local fdr* (for microarray) and *q*-value (for RNA-Seq). Through differential co-expression (DCE) and preservation analysis, we studied the behavioral changes among the DEGs under normal and disease circumstances. All non-preserved modules of reasonable sizes are considered modules of interest and analyzed further down the pipeline. DEGs are considered candidates for potential biomarkers for ESCC when they are either: (a) hub genes in the modules of interest, or (b) Top Enriched DEG (TED), i.e., a DEG annotated to the most enriched GO term in all three GO databases as well as the most enriched KEGG pathway in their respective datasets. Our proposed framework was validated on two microarray datasets (GSE20347 and GSE23400) and one RNA-Sequencing dataset (GSE130078). From, 7, 3 and 8 modules of interest in GSE20347, GSE23400 and GSE130078 respectively, 124, 59 and 160 hub genes were identified. The consideration of 22, 18 and 16 TEDs detected by GSE20347, GSE23400 and GSE130078 respectively results in 146, 77 and 176 as candidates for potential biomarkers of ESCC. Biological relevance for each candidate to their respective datasets is analysed based on (a) annotation to enriched GO terms in the GO databases, (b) annotation to enriched KEGG pathways, and (c) if the candidate gene is a transcription factor in a gene regulatory network. Another very important criterion that we considered for a candidate gene to be a potential biomarker is previous literature that has either (a) established them as potential biomarkers for ESCC itself, or (b) established them as potential biomarkers for 5 other SCCs related to ESCC, namely, Oral SCC, Tongue SCC, Lung SCC, Head and Neck SCC and Laryngeal SCC.

Our method identified 4 candidate genes, *STAT1*, *HIF1A* , *DNMT3B* and *MCM7*, that are transcription factors (TFs), have strong biological relevance to their respective datasets and have previous literature works that establish their role as potential biomarkers in ESCC. Our method identified *GSK3B* , detected as DEG by both microarray datasets (GSE20347 and GSE23400), as a TED and has both strong literature evidence as potential biomarker of ESCC and we established its strong biological relevance to both microarray datasets. Similarly, nine (*HOMER3* , *PSMD4*, *PSTAT1*, *TFRC*, *MCL1*, *EPHA2*, *KPNA2*, *CKS2* and *PRMT1*), seven (*HLA-F*, *HLA-G*, *CXCL10*, *ISG15*, *PSMA3*, *FCGR2A* and *C3AR1*) and four (*CAV1*, *VEGFC*, *CDK4* and *MSH2*) candidates genes in GSE20347, GSE23400 and GSE130078 are established as potential biomarkers for ESCC. We further identified 3 (*PTMA*, *VGLL4* and *NONO*), 1 (*PLEK2*) and 2 (*HMGB3* and *ESRRG*) TFs in GSE20347, GSE23400 and GSE130078 respectively, that have strong literature evidence as potential biomarkers of ESCC, but have moderate evidence for biological relevance to their respective datasets, and thus can be regarded as probable potential biomarkers for ESCC. On the other side of the spectrum, the transcription factor *AR*, a TED that is identified as a DEG in both microarray datasets and *PSMC2* have strong biological relevance to their datasets, but have been identified as potential biomarkers for other SCC related to ESCC. They can also be considered probable potential biomarkers for ESCC, but need further in-depth analysis. For future work, there is scope for improvement in the framework in general and consensus function specifically, towards detection of a smaller number of DEGs with minimum loss of relevant information.

Declarations

Conflict of interest On the behalf of all authors, the corresponding authors states that there is no conflict of interest.

References

1. Smyth GK, limma, Linear Models for Microarray Data. In: Gentleman R, Carey VJ, Huber W, Irizarry RA, Dudoit S, editors. Bioinformatics and Computational Biology Solutions Using R and Bioconductor. New York, NY: Springer; 2005. p. 397–420. https://doi.org/10.1007/0-387-29362-0.
2. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci. 2001;98(9):5116–21. https://doi.org/10.1073/pnas.091062498.
3. Efron B, Tibshirani R, Storey JD, Tusher V. Empirical Bayes analysis of a microarray experiment. J Am Stat Assoc. 2001;96(456):1151–60. https://doi.org/10.1198/016214501753382129.
4. Law CW, Chen Y, Shi W, Smyth GK. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 2014;15(2):R29. https://doi.org/10.1186/gb-2014-15-2-r29.
5. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139–40. https://doi.org/10.1093/bioinformatics/btp616.
6. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550. https://doi.org/10.1186/s13059-014-0550-8.
7. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinform. 2008;9:559. https://doi.org/10.1186/1471-2105-9-559.
8. Ravasz E, Somera AL, Mongru DA, Oltvai ZN, Barabási AL. Hierarchical organization of modularity in metabolic networks. Science. 2002;297(5586):1551–5. https://doi.org/10.1126/science.1073374.
9. Langfelder P, Luo R, Oldham MC, Horvath S. Is My Network Module Preserved and Reproducible? PLoS Comput Biol. 2011;7(1): https://doi.org/10.1371/journal.pcbi.1001057.
10. Sherman BT, Lempicki RA, et al. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44–57. https://doi.org/10.1038/nprot.2008.211.
11. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis

of large gene lists. Nucleic Acids Res. 2009;37(1):1–13. https://doi.org/10.1093/nar/gkn923.
12. Bhagwat AS, Vakoc CR. Targeting Transcription Factors in Cancer. Trends Cancer. 2015;1(1):53–65. https://doi.org/10.1016/j.trecan.2015.07.001.
13. Zhu Y, Qi X, Yu C, Yu S, Zhang C, Zhang Y, et al. Identification of prothymosin alpha (PTMA) as a biomarker for esophageal squamous cell carcinoma (ESCC) by label-free quantitative proteomics and Quantitative Dot Blot (QDB). Clin Proteomics. 2019;16(1):12. https://doi.org/10.1186/s12014-019-9232-6.
14. Tang P, Jia R, Gong L, Sui Z, Xiao W, Yang Y, Gong L, Sui Z, Xiao W, Yang Y, Wu X and Yu Z, Zhang, H. High expression of PPFIA1 in human esophageal squamous cell carcinoma correlates with tumor progression and poor prognosis. Res Square; 2022. https://doi.org/10.21203/rs.3.rs-554718/v2.
15. Jiang W, Yao F, He J, Lv B, Fang W, Zhu W, He G, Chen J, He J. Downregulation of VGLL4 in the progression of esophageal squamous cell carcinoma. Tumor Biol 2015;36(2):1289–97. https://doi.org/10.1007/s13277-014-2701-7.
16. Shen TY, Mei LL, Qiu YT, Shi ZZ. Identification of candidate target genes of genomic aberrations in esophageal squamous cell carcinoma. Oncol Lett. 2016;12(4):2956–2961. https://doi.org/10.3892/ol.2016.4947.
17. Ma AG, Yu LM, Zhao H, Qin CW, Tian XY, Wang Q. PSMD4 regulates the malignancy of esophageal cancer cells by suppressing endoplasmic reticulum stress. Kaohsiung J Med Sci. 2019;35(10):591–597. https://doi.org/10.1002/kjm2.12093.
18. Chen WC, Chen MF, Lin PY. Significance of DNMT3b in oral cancer. PLoS One. 2014;9(3):e89956. https://doi.org/10.1371/journal.pone.0089956.
19. Liu B, Jia Y, Cao Y, Wu S, Jiang H, Sun X, et al. Overexpression of Phosphoserine Aminotransferase 1 (PSAT1) Predicts Poor Prognosis and Associates with Tumor Progression in Human Esophageal Squamous Cell Carcinoma. Cell Physiol Biochem. 2016;39(1):395–406.https://doi.org/10.1159/000445633 .
20. Cheng R, Zhu S, Guo S, Min L, Xing J, Guo Q, Li P, Zhang S. Downregulation of NONO induces apoptosis, suppressing growth and invasion in esophageal squamous cell carcinoma. Oncol Rep. 2018;39(6):2575–2583. https://doi.org/10.3892/or.2018.6334.
21. Wada S, Noguchi T, Takeno S, Kawahara K. PIK3CA and TFRC located in 3q are new prognostic factors in esophageal squamous cell carcinoma. Ann Surg Oncol. 2006;13(7):961–6. https://doi.org/10.1245/ASO.2006.08.006.
22. He JZ, Wu ZY, Wang SH, Ji X, Yang CX, Xu XE, et al. A decision tree-based combination of ezrin-interacting proteins to estimate the prognostic risk of patients with esophageal squamous cell carcinoma. Hum Pathol. 2017;66:115–125. https://doi.org/10.1016/j.humpath.2017.06.003.
23. Yu X, Li W, Xia Z, Xie L, Ma X, Liang Q, Liu L, Wang J, Zhou X, Yang Y, Liu H. Targeting MCL-1 sensitizes human esophageal squamous cell carcinoma cells to cisplatin-induced apoptosis. BMC Cancer. 2017;17(1):449. https://doi.org/10.1186/s12885-017-3442-y.
24. Yang X, Han B, He Z, Zhang Y, Lin K, Su H, Hosseini DK, Sun H, Yang M, Chen X. RNA-Binding Proteins CLK1 and POP7 as Biomarkers for Diagnosis and Prognosis of Esophageal Squamous Cell Carcinoma. Front Cell Dev Biol. 2021;9:715027. https://doi.org/10.3389/fcell.2021.715027.
25. Qiu YT, Wang WJ, Zhang B, Mei LL, Shi ZZ. MCM7 amplification and overexpression promote cell proliferation, colony formation and migration in esophageal squamous cell carcinoma by activating the AKT1/mTOR signaling pathway. Oncol Rep. 2017;37(6):3590–3596. https://doi.org/10.3892/or.2017.5614.
26. Choy B, LaLonde A, Que J, Wu T, Zhou Z. MCM4 and MCM7, potential novel proliferation markers, significantly correlated with Ki-67, Bmi1, and cyclin E expression in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions. Hum Pathol. 2016;57:126–135. https://doi.org/10.1016/j.humpath.2016.07.013.
27. Zhong X, Chen X, Guan X, Zhang H, Ma Y, Zhang S, Wang E, Zhang L, Han Y. Overexpression of G9a and MCM7 in oesophageal squamous cell carcinoma is associated with poor prognosis. Histopathology. 2015;66(2):192–200. https://doi.org/10.1111/his.12456.
28. Miyazaki T, Kato H, Fukuchi M, Nakajima M, Kuwano H. EphA2 overexpression correlates with poor prognosis in esophageal squamous cell carcinoma. Int J Cancer. 2003;103(5):657–63. https://doi.org/10.1002/ijc.10860.
29. Ma S, Zhao X. KPNA2 is a promising biomarker candidate for esophageal squamous cell carcinoma and correlates with cell proliferation. Oncol Rep. 2014;32(4):1631–7. https://doi.org/10.3892/or.2014.3381.
30. Sakai M, Sohda M, Miyazaki T, Suzuki S, Sano A, Tanaka N, et al. Significance of karyopherin-α 2 (KPNA2) expression in esophageal squamous cell carcinoma. Anticancer Res. 2010;30(3):851–6.
31. Wang L, Liu G, Bolor-Erdene E, Li Q, Mei Y, Zhou L. Identification of KIF4A as a prognostic biomarker for esophageal squamous cell carcinoma. Aging (Albany NY). 2021;13(21):24050–24070. https://doi.org/10.18632/aging.203585.
32. Sun X, Chen P, Chen X, Yang W, Chen X, Zhou W, Chen X, Zhou W, Huang D, Cheng Y. KIF4A enhanced cell proliferation and migration via Hippo signaling and predicted a poor prognosis in esophageal squamous cell carcinoma. Thorac Cancer. 2021;12(4):512–524. https://doi.org/10.1111/1759-7714.13787.
33. Kita Y, Nishizono Y, Okumura H, Uchikado Y, Sasaki K, Matsumoto M, Setoyama T, Tanoue K, Omoto I, Mori S, Owaki T, Ishigami S, Nakagawa H, Tanaka F, Mimori K, Mori M, Natsugoe S. Clinical and biological impact of cyclin-dependent kinase subunit 2 in esophageal squamous cell carcinoma. Oncol Rep. 2014; 31(5):1986–92. https://doi.org/10.3892/or.2014.3062.
34. Zheng X, Zhao Y, Wang X, Li Y, Wang R, Jiang Y, Gong T, Li M, Sun L, Hong L, Li X, Liang J, Luo G, Jin B, Yang J, Zhang H, Fan D. Decreased expression of CIAPIN1 is correlated with poor prognosis in patients with esophageal squamous cell carcinoma. Dig Dis Sci. 2010; 55(12):3408–14. https://doi.org/10.1007/s10620-010-1212-7.
35. Zhao Y, Lu Q, Li C, Wang X, Jiang L, Huang L, Wang C, Chen H. PRMT1 regulates the tumour-initiating properties of esophageal squamous cell carcinoma through histone H4 arginine methylation coupled with transcriptional activation. Cell Death Dis. 2019; 10(5):359. https://doi.org/10.1038/s41419-019-1595-0.
36. Zhou W, Yue H, Li C, Chen H, Yuan Y. Protein arginine methyltransferase 1 promoted the growth and migration of cancer cells in esophageal squamous cell carcinoma. Tumour Biol. 2016; 37(2):2613–9. https://doi.org/10.1007/s13277-015-4098-3.
37. Zhang X, Lin A, Zhang JG, Bao WG, Xu DP, Ruan YY, Yan WH. Alteration of HLA-F and HLA I antigen expression in the tumor is associated with survival in patients with esophageal squamous cell carcinoma. Int J Cancer. 2013; 132(1):82–9. https://doi.org/10.1002/ijc.27621.
38. Yie SM, Yang H, Ye SR, Li K, Dong DD, Lin XM. Expression of HLA-G is associated with prognosis in esophageal squamous cell carcinoma. Am J Clin Pathol. 2007;128(6):1002–9. https://doi.org/10.1309/JNCW1QLDFB6AM9WE.
39. Sato Y, Motoyama S, Nanjo H, Wakita A, Yoshino K, Sasaki T, Nagaki Y, Liu J, Imai K, Saito H, Minamiya Y. CXCL10

Expression Status is Prognostic in Patients with Advanced Thoracic Esophageal Squamous Cell Carcinoma. Ann Surg Oncol. 2016;23(3):936-42. https://doi.org/10.1245/s10434-015-4909-1.
40. Yuan H, Zhou W, Yang Y, Xue L, Liu L, Song Y. ISG15 promotes esophageal squamous cell carcinoma tumorigenesis via c-MET/Fyn/β-catenin signaling pathway. Exp Cell Res. 2018; 367(1):47-55. https://doi.org/10.1016/j.yexcr.2018.03.017.
41. Yu H, Yao J, Du M, Ye J, He X, Yin L. CDKN3 promotes cell proliferation, invasion and migration by activating the AKT signaling pathway in esophageal squamous cell carcinoma. Oncol Lett. 2020;19(1):542-548. https://doi.org/10.3892/ol.2019.11077.
42. Wang W, Liao K, Guo HC, Zhou S, Yu R, Liu Y, Pan Y, Pu J. Integrated transcriptomics explored the cancer-promoting genes CDKN3 in esophageal squamous cell cancer. J Cardiothorac Surg. 2021;16(1):148. https://doi.org/10.1186/s13019-021-01534-7.
43. Liu J, Min L, Zhu S, Guo Q, Li H, Zhang Z, Zhao Y, Xu C, Zhang S. Cyclin-Dependent Kinase Inhibitor 3 Promoted Cell Proliferation by Driving Cell Cycle from G1 to S Phase in Esophageal Squamous Cell Carcinoma. J Cancer. 2019; 10(8):1915-1922. https://doi.org/10.7150/jca.27053.
44. Hu J, Li R, Miao H, Wen Z. Identification of key genes for esophageal squamous cell carcinoma via integrated bioinformatics analysis and experimental confirmation. J Thorac Dis. 2020; 12(6):3188-3199. https://doi.org/10.21037/jtd.2020.01.33.
45. Liu ZC, Cao K, Xiao ZH, Qiao L, Wang XQ, Shang B, Jia Y, Wang Z. VRK1 promotes cisplatin resistance by up-regulating c-MYC via c-Jun activation and serves as a therapeutic target in esophageal squamous cell carcinoma. Oncotarget. 2017; 8(39):65642-65658. https://doi.org/10.18632/oncotarget.20020.
46. Liu J, Shao J, Zhang C, Qin G, Liu J, Li M, Wu P, Zhao X, Zhang Y. Immuno-oncological role of 20S proteasome alpha-subunit 3 in aggravating the progression of esophageal squamous cell carcinoma. Eur J Immunol. 2022; 52(2):338-351. https://doi.org/10.1002/eji.202149441.
47. Wang F, Zhang C, Cheng H, Liu C, Lu Z, Zheng S, Wang S, Sun N, He J. TGF-β-induced PLEK2 promotes metastasis and chemoresistance in oesophageal squamous cell carcinoma by regulating LCN2. Cell Death Dis. 2021 Oct 2;12(10):901. https://doi.org/10.1038/s41419-021-04155-z.
48. Qu J, Zhao Q, Yang L, Ping Y, Zhang K, Lei Q, Liu F, Zhang Y. Identification and characterization of prognosis-related genes in the tumor microenvironment of esophageal squamous cell carcinoma. Int Immunopharmacol. 2021; 96:107616. https://doi.org/10.1016/j.intimp.2021.107616.
49. Zhang Y, Molavi O, Su M, Lai R. The clinical and biological significance of STAT1 in esophageal squamous cell carcinoma. BMC Cancer. 2014; 14:791. https://doi.org/10.1186/1471-2407-14-791 .
50. Shao N, Han Y, Song L, Song W. Clinical significance of hypoxia-inducible factor 1α, and its correlation with p53 and vascular endothelial growth factor expression in resectable esophageal squamous cell carcinoma. J Cancer Res Ther. 2020;16(2):269-275. https://doi.org/10.4103/jcrt.JCRT_781_19.
51. Hu X, Lin J, Jiang M, He X, Wang K, Wang W, Hu C, Shen Z, He Z, Lin H, Wu D, Wang M. HIF-1α Promotes the Metastasis of Esophageal Squamous Cell Carcinoma by Targeting SP1. J Cancer. 2020; 11(1):229-240. https://doi.org/10.7150/jca.35537.
52. Bolidong D, Domoto T, Uehara M, Sabit H, Okumura T, Endo Y, Nakada M, Ninomiya I, Miyashita T, Wong RW, Minamoto T. Potential therapeutic effect of targeting glycogen synthase kinase 3β in esophageal squamous cell carcinoma. Sci Rep. 2020; 10(1):11807. https://doi.org/10.1038/s41598-020-68713-9.
53. Gao S, Li S, Duan X, Gu Z, Ma Z, Yuan X, Feng X, Wang H. Inhibition of glycogen synthase kinase 3 beta (GSK3β) suppresses the progression of esophageal squamous cell carcinoma by modifying STAT3 activity. Mol Carcinog. 2017; 56(10):2301-2316. https://doi.org/10.1002/mc.22685.
54. Kato K, Hida Y, Miyamoto M, Hashida H, Shinohara T, Itoh T, Okushiba S, Kondo S, Katoh H. Overexpression of caveolin-1 in esophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. Cancer. 2002; 94(4):929-33. https://doi.org/10.1002/cncr.10329.
55. Ando T, Ishiguro H, Kimura M, Mitsui A, Mori Y, Sugito N, Tomoda K, Mori R, Harada K, Katada T, Ogawa R, Fujii Y, Kuwabara Y. The overexpression of caveolin-1 and caveolin-2 correlates with a poor prognosis and tumor progression in esophageal squamous cell carcinoma. Oncol Rep. 2007;18(3):601-9.
56. Lu F, Chen W, Jiang T, Cheng C, Wang B, Lu Z, Huang G, Qiu J, Wei W, Yang M, Huang X. Expression profile, clinical significance and biological functions of IGF2BP2 in esophageal squamous cell carcinoma. Exp Ther Med. 2022; 23(4):252. https://doi.org/10.3892/etm.2022.11177.
57. Shu W, Lin Y, Yan Y, Sun Y, Wu X, Cao Q. IGF2BP2 Promotes the Proliferation, Invasion and Migration of Esophageal Carcinoma Cells via Activation of the PI3K/AKT/EMT Signaling Pathway. Research Square; 2021. https://doi.org/10.21203/rs.3.rs-711778/v1.
58. Tanaka T, Ishiguro H, Kuwabara Y, Kimura M, Mitsui A, Katada T, Shiozaki M, Naganawa Y, Fujii Y, Takeyama H. Vascular endothelial growth factor C (VEGF-C) in esophageal cancer correlates with lymph node metastasis and poor patient prognosis. J Exp Clin Cancer Res. 2010; 29(1):83. https://doi.org/10.1186/1756-9966-29-83.
59. Kimura Y, Watanabe M, Ohga T, Saeki H, Kakeji Y, Baba H, Maehara Y. Vascular endothelial growth factor C expression correlates with lymphatic involvement and poor prognosis in patients with esophageal squamous cell carcinoma. Oncol Rep. 2003; 10(6):1747-51. https://doi.org/10.3892/or.10.6.1747.
60. Feng YF, Lei YY, Lu JB, Xi SY, Zhang Y, Huang QT, Wu QL, Wang F. RIT1 suppresses esophageal squamous cell carcinoma growth and metastasis and predicts good prognosis. Cell Death Dis. 2018; 9(11):1085. https://doi.org/10.1038/s41419-018-0979-x.
61. Zhou Y, Wang L, Ban X, Zeng T, Zhu Y, Li M, Guan XY, Li Y. DHRS2 inhibits cell growth and motility in esophageal squamous cell carcinoma. Oncogene. 2018; 37(8):1086-1094. https://doi.org/10.1038/onc.2017.383.
62. Li Y, Chen L, Nie CJ, Zeng TT, Liu H, Mao X, Qin Y, Zhu YH, Fu L, Guan XY. Downregulation of RBMS3 is associated with poor prognosis in esophageal squamous cell carcinoma. Cancer Res. 2011; 71(19):6106-15. https://doi.org/10.1158/0008-5472.CAN-10-4291.
63. Wang X, Liu Y, Leng X, Cao K, Sun W, Zhu J, Ma J. UBE2T Contributes to the Prognosis of Esophageal Squamous Cell Carcinoma. Pathol. Oncol. Res. 2021;27: https://doi.org/10.3389/pore.2021.632531.
64. Gao J, Zou Z, Gao J, Zhang H, Lin Z, Zhang Y, Luo X, Liu C, Xie J, Cai C. Increased expression of HMGB3: a novel independent prognostic marker of worse outcome in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2015; 8(1):345-52.
65. Huang J, Wang X, Zhang X, Chen W, Luan L, Song Q, Wang H, Liu J, Xu L, Xu Y, Shen L, Tan L, Jiang D, Su J, Hou Y. CDK4 Amplification in Esophageal Squamous Cell Carcinoma Associated With Better Patient Outcome. Front Genet. 2021; 12:616110. https://doi.org/10.3389/fgene.2021.616110.

66. Ling ZQ, Zhao Q, Zhou SL, Mao WM. MSH2 promoter hypermethylation in circulating tumor DNA is a valuable predictor of disease-free survival for patients with esophageal squamous cell carcinoma. Eur J Surg Oncol. 2012; 38(4):326-32. https://doi.org/10.1016/j.ejso.2012.01.008.
67. Xu Y, Wang N, Liu R, Lv H, Li Z, Zhang F, Gai C, Tian Z. Epigenetic Study of Esophageal Carcinoma Based on Methylation, Gene Integration and Weighted Correlation Network Analysis. Onco Targets Ther. 2021; 14:3133-3149. https://doi.org/10.2147/OTT.S298620.
68. Chen X, Lu B, Ma Q, Ji CD, Li JZ. EphA3 inhibits migration and invasion of esophageal cancer cells by activating the mesenchymal-epithelial transition process. Int J Oncol. 2019; 54(2):722-732. https://doi.org/10.3892/ijo.2018.4639.
69. Luo LL, Zhao L, Wang YX, Tian XP, Xi M, Shen JX, He LR, Li QQ, Liu SL, Zhang P, Xie D, Liu MZ. Insulin-like growth factor binding protein-3 is a new predictor of radiosensitivity on esophageal squamous cell carcinoma. Sci Rep. 2015; 5(1):17336. https://doi.org/10.1038/srep17336.
70. Zhao L, Li QQ, Zhang R, Xi M, Liao YJ, Qian D, He LR, Zeng YX, Xie D, Liu MZ. The overexpression of IGFBP-3 is involved in the chemosensitivity of esophageal squamous cell carcinoma cells to nimotuzumab combined with cisplatin. Tumour Biol. 2012; 33(4):1115-23. https://doi.org/10.1007/s13277-012-0352-0.
71. Luo LL, Zhao L, Xi M, He LR, Shen JX, Li QQ, Liu SL, Zhang P, Xie D, Liu MZ. Association of insulin-like growth factor-binding protein-3 with radiotherapy response and prognosis of esophageal squamous cell carcinoma. Chin J Cancer. 2015; 34(11):514-21. https://doi.org/10.1186/s40880-015-0046-2.
72. Wang X, Cheng G, Zhang T, Deng L, Xu K, Xu X, Wang W, Zhou Z, Feng Q, Chen D, Bi N, Wang L. CHST15 promotes the proliferation of TE-1 cells via multiple pathways in esophageal cancer. Oncol Rep. 2020; 43(1):75-86. https://doi.org/10.3892/or.2019.7395.
73. Tripathi SC, Matta A, Kaur J, Grigull J, Chauhan SS, Thakar A, Shukla NK, Duggal R, Choudhary AR, Dattagupta S, Sharma MC, Ralhan R, Siu KW. Overexpression of prothymosin alpha predicts poor disease outcome in head and neck cancer. PLoS One. 2011; 6(5):e19213. https://doi.org/10.1371/journal.pone.0019213.
74. Tan KD, Zhu Y, Tan HK, Rajasegaran V, Aggarwal A, Wu J, Wu HY, Hwang J, Lim DT, Soo KC, Tan P. Amplification and overexpression of PPFIA1, a putative 11q13 invasion suppressor gene, in head and neck squamous cell carcinoma. Genes Chromosomes Cancer. 2008; 47(4):353-62. https://doi.org/10.1002/gcc.20539.
75. Al-Rawi NH, Merza MS, Ghazi AM, et al. PIK3CB and K-ras in oral squamous Cell carcinoma. A possible cross-talk! J Orofac Sci. 2014;6(2):99.
76. Liang Y, Lei Y, Du M, Liang M, Liu Z, Li X, Gao Y. The increased expression and aberrant methylation of SHC1 in non-small cell lung cancer: Integrative analysis of clinical and bioinformatics databases. J Cell Mol Med. 2021; 25(14):7039-7051. https://doi.org/10.1111/jcmm.16717.
77. Xue H, Lu J, Yuan R, Liu J, Liu Y, Wu K, Wu J, Du J, Shen B. Knockdown of CLIC4 enhances ATP-induced HN4 cell apoptosis through mitochondrial and endoplasmic reticulum pathways. Cell Biosci. 2016;6:5. https://doi.org/10.1186/s13578-016-0070-1.
78. Chen MF, Lu MS, Lin PY, Chen PT, Chen WC, Lee KD. The role of DNA methyltransferase 3b in esophageal squamous cell carcinoma. Cancer. 2012;118(16):4074-89. https://doi.org/10.1002/cncr.26736.
79. Liu Z, Wang L, Wang LE, Sturgis EM, Wei Q. Polymorphisms of the DNMT3B gene and risk of squamous cell carcinoma of the head and neck: a case-control study. Cancer Lett. 2008; 268(1):158-65. https://doi.org/10.1016/j.canlet.2008.03.034.
80. Ohmura G, Tsujikawa T, Yaguchi T, Kawamura N, Mikami S, Sugiyama J, Nakamura K, Kobayashi A, Iwata T, Nakano H, Shimada T, Hisa Y, Kawakami Y. Aberrant Myosin 1b Expression Promotes Cell Migration and Lymph Node Metastasis of HNSCC. Mol Cancer Res. 2015; 13(4):721-31. https://doi.org/10.1158/1541-7786.MCR-14-0410.
81. Liang J, Sun Z. Overexpression of membranal SLC3A2 regulates the proliferation of oral squamous cancer cells and affects the prognosis of oral cancer patients. J Oral Pathol Med. 2021; 50(4):371-377. https://doi.org/10.1111/jop.13132.
82. Ding X, Sun J, Wang L, Li G, Shen Y, Zhou X, Chen W. Overexpression of SENP5 in oral squamous cell carcinoma and its association with differentiation. Oncol Rep. 2008 Nov;20(5):1041-5. https://doi.org/10.3892/or_00000107.
83. Ueda S, Hashimoto K, Miyabe S, Hasegawa S, Goto M, Shimizu D, Oh-Iwa I, Shimozato K, Nagao T, Nomoto S. Salivary NUS1 and RCN1 Levels as Biomarkers for Oral Squamous Cell Carcinoma Diagnosis. In Vivo. 2020; 34(5):2353-2361. https://doi.org/10.21873/invivo.12048.
84. Zhu MH, Ji SL, Zhang CY, Cui L, Xiong L, Zheng HL. DNA microarray reveals ZNF195 and SBF1 are potential biomarkers for gemcitabine sensitivity in head and neck squamous cell carcinoma cell lines. Int J Clin Exp Pathol. 2014;7(4):1514-23.
85. Maji S, Samal SK, Pattanaik L, Panda S, Quinn BA, Das SK, Sarkar D, Pellecchia M, Fisher PB, Dash R. Mcl-1 is an important therapeutic target for oral squamous cell carcinomas. Oncotarget. 2015; 6(18):16623-37. https://doi.org/10.18632/oncotarget.3932.
86. Feng CJ, Li HJ, Li JN, Lu YJ, Liao GQ. Expression of Mcm7 and Cdc6 in oral squamous cell carcinoma and precancerous lesions. Anticancer Res. 2008; 28(6A):3763-9.
87. Ma H, Li L, Jia L, Gong A, Wang A, Zhang L, Gu M, Tang G. POM121 is identified as a novel prognostic marker of oral squamous cell carcinoma. J Cancer. 2019; 10(19):4473-4480. https://doi.org/10.7150/jca.33368.
88. Wang Z, Xiong H, Zuo Y, Hu S, Zhu C, Min A. PSMC2 knockdown inhibits the progression of oral squamous cell carcinoma by promoting apoptosis via PI3K/Akt pathway. Cell Cycle. 2022; 21(5):477-488. https://doi.org/10.1080/15384101.2021.2021722.
89. Faoro L, Singleton PA, Cervantes GM, Lennon FE, Choong NW, Kanteti R, Ferguson BD, Husain AN, Tretiakova MS, Ramnath N, Vokes EE, Salgia R. EphA2 mutation in lung squamous cell carcinoma promotes increased cell survival, cell invasion, focal adhesions, and mammalian target of rapamycin activation. J Biol Chem. 2010; 285(24):18575-85. https://doi.org/10.1074/jbc.M109.075085.
90. Liu Y, Zhang X, Qiu Y, Huang D, Zhang S, Xie L, Qi L, Yu C, Zhou X, Hu G, Tian Y. Clinical significance of EphA2 expression in squamous-cell carcinoma of the head and neck. J Cancer Res Clin Oncol. 2011; 137(5):761-9. https://doi.org/10.1007/s00432-010-0936-2.
91. Rivera RS, Gunduz M, Nagatsuka H, Gunduz E, Cengiz B, Fukushima K, Beder LB, Pehlivan D, Yamanaka N, Shimizu K, Nagai N. Involvement of EphA2 in head and neck squamous cell carcinoma: mRNA expression, loss of heterozygosity and immunohistochemical studies. Oncol Rep. 2008;19(5):1079-84. https://doi.org/10.3892/or.19.5.1079.
92. Lin F, Gao L, Su Z, Cao X, Zhan Y, Li Y, Zhang B. Knockdown of KPNA2 inhibits autophagy in oral squamous cell carcinoma cell lines by blocking p53 nuclear translocation. Oncol Rep. 2018; 40(1):179-194. https://doi.org/10.3892/or.2018.6451.

93. Minakawa Y, Kasamatsu A, Koike H, Higo M, Nakashima D, Kouzu Y, Sakamoto Y, Ogawara K, Shiiba M, Tanzawa H, Uzawa K. Kinesin family member 4A: a potential predictor for progression of human oral cancer. PLoS One. 2013; 8(12):e85951. https://doi.org/10.1371/journal.pone.0085951.
94. Dong Y, Lu S, Wang Z, Liu L. CCTs as new biomarkers for the prognosis of head and neck squamous cancer. Open Med (Wars). 2020; 15(1):672-688. https://doi.org/10.1515/med-2020-0114.
95. Gao F, Li C, Zhao X, Xie J, Fang G, Li Y. CKS2 modulates cell-cycle progression of tongue squamous cell carcinoma cells partly via modulating the cellular distribution of DUTPase. J Oral Pathol Med. 2021; 50(2):175-182. https://doi.org/10.1111/jop.13116.
96. Chuang CY, Chang CP, Lee YJ, Lin WL, Chang WW, Wu JS, Cheng YW, Lee H, Li C. PRMT1 expression is elevated in head and neck cancer and inhibition of protein arginine methylation by adenosine dialdehyde or PRMT1 knockdown downregulates proliferation and migration of oral cancer cells. Oncol Rep. 2017; 38(2):1115-1123. https://doi.org/10.3892/or.2017.5737.
97. Wu T, Jiao Z, Li Y, Su X, Yao F, Peng J, Chen W, Yang A. HPRT1 Promotes Chemoresistance in Oral Squamous Cell Carcinoma via Activating MMP1/PI3K/Akt Signaling Pathway. Cancers (Basel). 2022; 14(4):855. https://doi.org/10.3390/cancers14040855.
98. Ahmadi M, Eftekhari Kenzerki M, Akrami SM, Pashangzadeh S, Hajiesmaeili F, Rahnavard S, Habibipour L, Saffarzadeh N, Mousavi P. Overexpression of HPRT1 is associated with poor prognosis in head and neck squamous cell carcinoma. FEBS Open Bio. 2021;11(9):2525-2540. https://doi.org/10.1002/2211-5463.13250.
99. Zhang G, Zhao X, Liu W. NEDD4L inhibits glycolysis and proliferation of cancer cells in oral squamous cell carcinoma by inducing ENO1 ubiquitination and degradation. Cancer Biol Ther. 2022; 23(1):243-253. https://doi.org/10.1080/15384047.2022.2054244.
100. Acker F, Stratmann J, Aspacher L, Nguyen NTT, Wagner S, Serve H, Wild PJ, Sebastian M. KRAS Mutations in Squamous Cell Carcinomas of the Lung. Front Oncol. 2021;11: https://doi.org/10.3389/fonc.2021.788084.
101. Vallejo-Díaz J, Olazabal-Morán M, Cariaga-Martínez AE, Pajares MJ, Flores JM, Pio R, Montuenga LM, Carrera AC. Targeted depletion of PIK3R2 induces regression of lung squamous cell carcinoma. Oncotarget. 2016; 7(51):85063-85078. https://doi.org/10.18632/oncotarget.13195.
102. Attaran N, Gu X, Coates PJ, Fåhraeus R, Boldrup L, Wilms T, Wang L, Sgaramella N, Zborayova K, Nylander K. Downregulation of TAP1 in Tumor-Free Tongue Contralateral to Squamous Cell Carcinoma of the Oral Tongue, an Indicator of Better Survival. Int J Mol Sci. 2020; 21(17):6220. https://doi.org/10.3390/ijms21176220.
103. Pidugu VK, Wu MM, Yen AH, Pidugu HB, Chang KW, Liu CJ, Lee TC. IFIT1 and IFIT3 promote oral squamous cell carcinoma metastasis and contribute to the anti-tumor effect of gefitinib via enhancing p-EGFR recycling. Oncogene. 2019;38(17):3232–247. https://doi.org/10.1038/s41388-018-0662-9.
104. Lee TC, Pidugu VK, Wu MM, Liu CJ. IFIT1 and IFIT3 modulate the drug response via enhancing EGFR signaling and in human oral squamous cell carcinoma cells. In: Proceedings for Annual Meeting of The Japanese Pharmacological Society WCP2018 (The 18th World Congress of Basic and Clinical Pharmacology), pp. PO4–6, 2018, Japanese Pharmacological Society.
105. Sarmah N, Baruah MN, Baruah S. Immune Modulation in HLA-G Expressing Head and Neck Squamous Cell Carcinoma in Relation to Human Papilloma Virus Positivity: A Study From Northeast India. Front Oncol. 2019;9:58. https://doi.org/10.3389/fonc.2019.00058.
106. Bora M, Sarmah N, Das B, Baruah MN, Deka G, Hazarika SG, Baruah S. A comparative study on regulation of HLA-G expression in bad obstetric history and in head and neck squamous cell carcinoma from Northeast India. Human Immunol. 2022;83(5):453–7. https://doi.org/10.1016/j.humimm.2022.02.006.
107. Imani R, Seyedmajidi M, Ghasemi N, Moslemi D, Shafaee S, Bijani A. HLA-G Expression is Associated with an Unfavorable Prognosis of Oral Squamous Cell Carcinoma. Asian Pac J Cancer Prev. 2018;19(9):2527–33https://doi.org/10.22034/APJCP.2018.19.9.2527.
108. Ou D, Wu Y. The prognostic and clinical significance of IFI44L aberrant downregulation in patients with oral squamous cell carcinoma. BMC Cancer. 2021;21(1):1327. https://doi.org/10.1186/s12885-021-09058-y.
109. Rentoft M, Coates PJ, Loljung L, Wilms T, Laurell G, Nylander K. Expression of CXCL10 is associated with response to radiotherapy and overall survival in squamous cell carcinoma of the tongue. Tumour Biol. 2014;35(5):4191–8. https://doi.org/10.1007/s13277-013-1549-6.
110. Li Y, Wu T, Gong S, Zhou H, Yu L, Liang M, Shi R, Wu Z, Zhang J, Li S. Analysis of the Prognosis and Therapeutic Value of the CXC Chemokine Family in Head and Neck Squamous Cell Carcinoma. Front Oncol. 2021;10: https://doi.org/10.3389/fonc.2020.570736.
111. Li H, Yang LL, Wu CC, Xiao Y, Mao L, Chen L, Zhang WF, Sun ZJ. Expression and Prognostic Value of IFIT1 and IFITM3 in Head and Neck Squamous Cell Carcinoma. Am J Clin Pathol. 2020;153(5):618–29. https://doi.org/10.1093/ajcp/aqz205.
112. Laljee RP, Muddaiah S, Salagundi B, Cariappa PM, Indra AS, Sanjay V, Ramanathan A. Interferon stimulated gene-ISG15 is a potential diagnostic biomarker in oral squamous cell carcinomas. Asian Pac J Cancer Prev. 2013;14(2):1147–50. https://doi.org/10.7314/apjcp.2013.14.2.1147.
113. Zhang Q, He Y, Nie M, Cai W. Roles of miR-138 and ISG15 in oral squamous cell carcinoma. Exp Ther Med. 2017;14(3):2329–34. https://doi.org/10.3892/etm.2017.4720.
114. Santos CR, Rodríguez-Pinilla M, Vega FM, Rodríguez-Peralto JL, Blanco S, Sevilla A, Valbuena A, Hernández T, van Wijnen AJ, Li F, de Alava E, Sánchez-Céspedes M, Lazo PA. VRK1 signaling pathway in the context of the proliferation phenotype in head and neck squamous cell carcinoma. Mol Cancer Res. 2006;4(3):177–85. https://doi.org/10.1158/1541-7786.MCR-05-0212.
115. Cao X, Luan K, Yang J, Huang Y. Targeting lncRNA PSMA3-AS1, a Prognostic Marker, Suppresses Malignant Progression of Oral Squamous Cell Carcinoma. Dis Markers. 2021;2021:3138046. https://doi.org/10.1155/2021/3138046.
116. Wang J, Sun Z, Wang J, Tian Q, Huang R, Wang H, Wang X, Han F. Expression and prognostic potential of PLEK2 in head and neck squamous cell carcinoma based on bioinformatics analysis. Cancer Med. 2021;10(18):6515–33. https://doi.org/10.1002/cam4.4163.
117. Troiano G, Caponio VCA, Adipietro I, Tepedino M, Santoro R, Laino L, Lo Russo L, Cirillo N, Lo Muzio L. Prognostic significance of CD68+ and CD163+ tumor associated macrophages in head and neck squamous cell carcinoma: A systematic review and meta-analysis. Oral Oncol. 2019;93:66–75. https://doi.org/10.1016/j.oraloncology.2019.04.019.
118. Kubota K, Moriyama M, Furukawa S, Rafiul HASM, Maruse Y, Jinno T, Tanaka A, Ohta M, Ishiguro N, Yamauchi M, Sakamoto M, Maehara T, Hayashida JN, Kawano S, Kiyoshima T, Nakamura S. CD163+CD204+ tumor-associated macrophages contribute to T cell regulation via interleukin-10 and

PD-L1 production in oral squamous cell carcinoma. Sci Rep. 2017;7(1):1755. https://doi.org/10.1038/s41598-017-01661-z.
119. He KF, Zhang L, Huang CF, Ma SR, Wang YF, Wang WM, Zhao ZL, Liu B, Zhao YF, Zhang WF, Sun ZJ. CD163+ tumor-associated macrophages correlated with poor prognosis and cancer stem cells in oral squamous cell carcinoma. Biomed Res Int. 2014;2014: 838632. https://doi.org/10.1155/2014/838632.
120. Zhang X, Dong Y, Zhao M, Ding L, Yang X, Jing Y, Song Y, Chen S, Hu Q, Ni Y. ITGB2-mediated metabolic switch in CAFs promotes OSCC proliferation by oxidation of NADH in mitochondrial oxidative phosphorylation system. Theranostics. 2020;10(26):12044–59. https://doi.org/10.7150/thno.47901.
121. Lu S, Li N, Peng Z, Lu Z, Tu X, Zhang W, Kang Y. Fc fragment of immunoglobulin G receptor IIa (FCGR2A) as a new potential prognostic biomarker of esophageal squamous cell carcinoma. Chin Med J (Engl). 2021;135(4):482–4. https://doi.org/10.1097/CM9.0000000000001776.
122. Dai Y, Chen W, Huang J, Cui T. FCGR2A Could Function as a Prognostic Marker and Correlate with Immune Infiltration in Head and Neck Squamous Cell Carcinoma. Biomed Res Int. 2021;2021:8874578. https://doi.org/10.1155/2021/8874578.
123. Magnes T, Melchardt T, Hufnagl C, Weiss L, Mittermair C, Neureiter D, Klieser E, Rinnerthaler G, Roesch S, Gaggl A, Greil R, Egle A. The influence of FCGR2A and FCGR3A polymorphisms on the survival of patients with recurrent or metastatic squamous cell head and neck cancer treated with cetuximab. Pharmacogenomics J. 2018; 18(3):474-479. https://doi.org/10.1038/tpj.2017.37.
124. Santos Md, Mercante AMdC, Louro ID, Gonçalves AJ, Carvalho MBd, da Silva EHT, et al. HIF1-Alpha Expression Predicts Survival of Patients with Squamous Cell Carcinoma of the Oral Cavity. PLoS ONE. 2012;7(9): e45228. https://doi.org/10.1371/journal.pone.0045228.
125. Zhou J, Huang S, Wang L, Yuan X, Dong Q, Zhang D, Wang X. Clinical and prognostic significance of HIF-1α overexpression in oral squamous cell carcinoma: a meta-analysis. World J Surg Oncol. 2017;15(1):104. https://doi.org/10.1186/s12957-017-1163-y.
126. Fillies T, Werkmeister R, van Diest PJ, Brandt B, Joos U, Buerger H. HIF1-alpha overexpression indicates a good prognosis in early stage squamous cell carcinomas of the oral floor. BMC Cancer. 2005;5:84. https://doi.org/10.1186/1471-2407-5-84.
127. Liang J, Zhang Z, Liang L, Shen Y, Ouyang K. HIF-1α regulated tongue squamous cell carcinoma cell growth via regulating VEGF expression in a xenograft model. Ann Transl Med. 2014;2(9):92. https://doi.org/10.3978/j.issn.2305-5839.2014.08.01.
128. Routray S. Caveolin-1 in oral squamous cell carcinoma microenvironment: an overview. Tumour Biol. 2014;35(10):9487–95. https://doi.org/10.1007/s13277-014-2482-z.
129. Jung AC, Ray AM, Ramolu L, Macabre C, Simon F, Noulet F, Blandin AF, Renner G, Lehmann M, Choulier L, Kessler H, Abecassis J, Dontenwill M, Martin S. Caveolin-1-negative head and neck squamous cell carcinoma primary tumors display increased epithelial to mesenchymal transition and prometastatic properties. Oncotarget. 2015;6(39):41884–901.https://doi.org/10.18632/oncotarget.6099.
130. Xue J, Chen H, Diao L, Chen X, Xia D. Expression of Caveolin-1 in tongue squamous cell carcinoma by quantum dots. Eur J Histochem. 2010;54(2):e20. https://doi.org/10.4081/ejh.2010.e20.
131. Zhou L, Li H, Cai H, Liu W, Pan E, Yu D, He S. Upregulation of IGF2BP2 Promotes Oral Squamous Cell Carcinoma Progression That Is Related to Cell Proliferation, Metastasis and Tumor-Infiltrating Immune Cells. Front Oncol. 2022;12:809589. https://doi.org/10.3389/fonc.2022.809589.
132. Wang X, Xu H, Zhou Z, Guo S, Chen R. IGF2BP2 maybe a novel prognostic biomarker in oral squamous cell carcinoma. Biosci Rep. 2022; 42(2):BSR20212119. https://doi.org/10.1042/BSR20212119.
133. Rapone B, Ferrara E. Vascular EndothelialGrowth Factor Expression in the PathologicalAngiogenesis in Oral Squamous Cell-Carcinoma. In: Sridharan G, SukumaranA, Al-Ostwani AEO, editors. Oral Diseases.Rijeka: IntechOpen; 2020
134. dos Santos Almeida A, Oliveira DT, Pereira MC, Faustino SE, Nonogaki S, Carvalho AL, Kowalski LP. Podoplanin and VEGF-C immunoexpression in oral squamous cell carcinomas: prognostic significance. Anticancer Res. 2013; 33(9):3969-76.
135. Na J, Zhou W, Yin M, Hu Y, Ma X. GNA13 promotes the proliferation and migration of lung squamous cell carcinoma cells through regulating the PI3K/AKT signaling pathway. Tissue Cell. 2022;76:101795. https://doi.org/10.1016/j.tice.2022.101795.
136. Liang YN, Liu Y, Meng Q, Li X, Wang F, Yao G, Wang L, Fu S, Tong D. RBMS3 is a tumor suppressor gene that acts as a favorable prognostic marker in lung squamous cell carcinoma. Med Oncol. 2015;32(2):459. https://doi.org/10.1007/s12032-014-0459-9.
137. Wang M, Xie T, Wu Y, Yin Q, Xie S, Yao Q, Xiong J, Zhang Q. Identification of RFC5 as a novel potential prognostic biomarker in lung cancer through bioinformatics analysis. Oncol Lett. 2018;16(4):4201-210. https://doi.org/10.3892/ol.2018.9221.
138. Simonik EA, Cai Y, Kimmelshue KN, Brantley-Sieders DM, Loomans HA, Andl CD, Westlake GM, Youngblood VM, Chen J, Yarbrough WG, Brown BT, Nagarajan L, Brandt SJ. LIM-Only Protein 4 (LMO4) and LIM Domain Binding Protein 1 (LDB1) Promote Growth and Metastasis of Human Head and Neck Cancer (LMO4 and LDB1 in Head and Neck Cancer). PLoS One. 2016;11(10):e0164804. https://doi.org/10.1371/journal.pone.0164804.
139. Kwong RA, Scarlett CJ, Kalish LH, Cole IE, Kench JG, Sum EY, Musgrove EA, Henshall SM, Lindeman GJ, Biankin AV, Visvader JE, Sutherland RL. LMO4 expression in squamous cell carcinoma of the anterior tongue. Histopathology. 2011;58(3):477–80. https://doi.org/10.1111/j.1365-2559.2011.03765.x.
140. van Caloen G, Machiels JP. Potential role of cyclin-dependent kinase 4/6 inhibitors in the treatment of squamous cell carcinoma of the head and neck. Curr Opin Oncol. 2019;31(3):122–30. https://doi.org/10.1097/CCO.0000000000000513.
141. Ku BM, Yi SY, Koh J, Bae YH, Sun JM, Lee SH, Ahn JS, Park K, Ahn MJ. The CDK4/6 inhibitor LY2835219 has potent activity in combination with mTOR inhibitor in head and neck squamous cell carcinoma. Oncotarget. 2016;7(12):14803–13.https://doi.org/10.18632/oncotarget.7543.
142. Lourenco GJ, Nogueira GAS, Oliveira CBM, Marson FAL, Lopes-Aguiar L, Costa EFD, Lima TRP, Liutti VT, Leal F, Santos VA, Rinck JA, Lima CSP, MLH1, MSH2, MSH3 and EXO1 polymorphisms and head and neck squamous cell carcinoma risk and prognosis. Journal of Clinical Oncology. 2015;33(15_suppl):6063–6063. https://doi.org/10.1200/jco.2015.33.15_suppl.6063.
143. Pereira CS, Oliveira MV, Barros LO, Bandeira GA, Santos SH, Basile JR, Guimarães AL, De Paula AM. Low expression of MSH2 DNA repair protein is associated with poor prognosis in head and neck squamous cell carcinoma. J Appl Oral Sci. 2013;21(5):416–21. https://doi.org/10.1590/1679-775720130206.
144. Wang Y, Lu Z, Hu Z, Zheng A, Wang F, Xu Y, et al. The upregulation of TANK-binding kinase 1 in head and neck squamous cell carcinoma. Differentiation. 2017;1(14):46–7.

145. Zhang H, Liu J, Fu X, Yang A. Identification of Key Genes and Pathways in Tongue Squamous Cell Carcinoma Using Bioinformatics Analysis. Med Sci Monit. 2017;23:5924–32.https://doi.org/10.12659/msm.905035.
146. Yuan Y, Xie X, Jiang Y, Wei Z, Wang P, Chen F, Li X, Sun C, Zhao H, Zeng X, Jiang L, Zhou Y, Dan H, Feng M, Liu R, Wang Z, Chen Q. LRP6 is identified as a potential prognostic marker for oral squamous cell carcinoma via MALDI-IMS. Cell Death Dis. 2017; 8(9):e3035. https://doi.org/10.1038/cddis.2017.433.
147. Shen Z, Hu Y, Zhou C, Yuan J, Xu J, Hao W, Deng H, Ye D. ESRRG promoter hypermethylation as a diagnostic and prognostic biomarker in laryngeal squamous cell carcinoma. J Clin Lab Anal. 2019;33(6):e22899. https://doi.org/10.1002/jcla.22899.
148. Fukushima R, Kasamatsu A, Nakashima D, Higo M, Fushimi K, Kasama H, Endo-Sakamoto Y, Shiiba M, Tanzawa H, Uzawa K. Overexpression of Translocation Associated Membrane Protein 2 Leading to Cancer-Associated Matrix Metalloproteinase Activation as a Putative Metastatic Factor for Human Oral Cancer. J Cancer. 2018;9(18):3326–3333. https://doi.org/10.7150/jca.25666.
149. Wang SH, Chen YL, Hsiao JR, Tsai FY, Jiang SS, Lee AY, Tsai HJ, Chen YW. Insulin-like growth factor binding protein 3 promotes radiosensitivity of oral squamous cell carcinoma cells via positive feedback on NF-κB/IL-6/ROS signaling. J Exp Clin Cancer Res. 2021;40(1):95. https://doi.org/10.1186/s13046-021-01898-7.
150. Sakata J, Hirosue A, Yoshida R, Matsuoka Y, Kawahara K, Arita H, Nakashima H, Yamamoto T, Nagata M, Kawaguchi S, Gohara S, Nagao Y, Yamana K, Toya R, Murakami R, Kuwahara Y, Fukumoto M, Nakayama H.Enhanced Expression of IGFBP-3 Reduces Radiosensitivity and Is Associated with Poor Prognosis in Oral Squamous Cell Carcinoma. Cancers (Basel). 2020;12(2):494. https://doi.org/10.3390/cancers12020494.
151. Hano K, Hatano K, Saigo C, Kito Y, Shibata T, Takeuchi T. Combination of Clptm1L and TMEM207 Expression as a Robust Prognostic Marker in Oral Squamous Cell Carcinoma. Frontiers in Oral Health. 2021; 2:638213. https://doi.org/10.3389/froh.2021.638213.
152. Hou Y, Xue F, Fu Y, Feng G, Wang R, Yuan H. CLPTM1L Is a Novel Putative Oncogene Promoting Tumorigenesis in Oral Squamous Cell Carcinoma. Cell Transplant. 2021;30:09636897211045970. https://doi.org/10.1177/0963689721104597O.
153. Iwasawa S, Yamano Y, Takiguchi Y, Tanzawa H, Tatsumi K, Uzawa K. Upregulation of thioredoxin reductase 1 in human oral squamous cell carcinoma. Oncol Rep. 2011;25(3):637–44. https://doi.org/10.3892/or.2010.1131.
154. Feng J, Han B, Yu C, Shen C, Wen Z. Co-expression Network Identification and Clinical Prognostic Evaluation of Hub Genes in Head and Neck Squamous Cell Carcinoma. Research Square; 2020. https://doi.org/10.21203/rs.3.rs-77378/v1.
155. Chien HT, Cheng SD, Chuang WY, Liao CT, Wang HM, Huang SF. Clinical Implications of FADD Gene Amplification and Protein Overexpression in Taiwanese Oral Cavity Squamous Cell Carcinomas. PLoS One. 2016;11(10):e0164870. https://doi.org/10.1371/journal.pone.0164870.
156. González-Moles MÁ, Ayén Á, González-Ruiz I, de Porras-Carrique T, González-Ruiz L, Ruiz-Ávila I, Ramos-García P. Prognostic and Clinicopathological Significance of FADD Upregulation in Head and Neck Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis. Cancers (Basel). 2020;12(9):2393. https://doi.org/10.3390/cancers12092393.
157. Rasamny JJ, Allak A, Krook KA, Jo VY, Policarpio-Nicolas ML, Sumner HM, Moskaluk CA, Frierson HF Jr, Jameson MJ. Cyclin D1 and FADD as biomarkers in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg. 2012;146(6):923–31. https://doi.org/10.1177/0194599811435052.
158. Knitz MW, Darragh LB, Bickett TE, Bhatia S, Bukkapatnam S, Gadwa J, Piper M, Corbo S, Nguyen D, Van Court B, Oweida A, Karam SD. Loss of cancer cell STAT1 improves response to radiation therapy and promotes T cell activation in head and neck squamous cell carcinoma. Cancer Immunol Immunother. 2022;71(5):1049–61. https://doi.org/10.1007/s00262-021-03059-3.
159. Xi S, Dyer KF, Kimak M, Zhang Q, Gooding WE, Chaillet JR, Chai RL, Ferrell RE, Zamboni B, Hunt J, Grandis JR. Decreased STAT1 expression by promoter methylation in squamous cell carcinogenesis. J Natl Cancer Inst. 2006;98(3):181–9. https://doi.org/10.1093/jnci/djj020.
160. Tomasovic-Loncaric C, Fucic A, Andabak A, Andabak M, Ceppi M, Bruzzone M, Vrdoljak D, Vucicevic-Boras V. Androgen Receptor as a Biomarker of Oral Squamous Cell Carcinoma Progression Risk. Anticancer Res. 2019;39(8):4285–289. https://doi.org/10.21873/anticanres.13593.
161. Liu X, Qing S, Che K, Li L, Liao X. Androgen receptor promotes oral squamous cell carcinoma cell migration by increasing EGFR phosphorylation. Onco Targets Ther. 2019;12:4245–252. https://doi.org/10.2147/OTT.S200718.
162. Kordi-Tamandani D, Sabers E, Jamali S, Rigi Ladiz M. ERK and RAF1 genes: analysis of methylation and expression profiles in patients with oral squamous cell carcinoma. Br J Biomed Sci. 2014;71(3):100–3. https://doi.org/10.1080/09674845.2014.11669972.
163. Mishra R, Nagini S, Rana A. Expression and inactivation of glycogen synthase kinase 3 alpha/beta and their association with the expression of cyclin D1 and p53 in oral squamous cell carcinoma progression. Mol Cancer. 2015;14(1):20. https://doi.org/10.1186/s12943-015-0300-x.
164. Matsuo FS, Andrade MF, Loyola AM, da Silva SJ, Silva MJB, Cardoso SV, et al. Pathologic significance of AKT, mTOR, and GSK3β proteins in oral squamous cell carcinoma-affected patients. Virchows Arch. 2018;472(6):983–997. https://doi.org/10.1007/s00428-018-2318-0.
165. Jain AP, Patel K, Pinto S, Radhakrishnan A, Nanjappa V, Kumar M, Raja R, Patil AH, Kumari A, Manoharan M, Karunakaran C, Murugan S, Keshava Prasad TS, Chang X, Mathur PP, Kumar P, Gupta R, Gupta R, Khanna-Gupta A, Sidransky D, Chatterjee A, Gowda H. MAP2K1 is a potential therapeutic target in erlotinib resistant head and neck squamous cell carcinoma. Sci Rep. 2019;9(1):18793. https://doi.org/10.1038/s41598-019-55208-5.
166. Patowary P, Bhattacharyya DK, Barah P. Identifying critical genes in esophageal squamous cell carcinoma using an ensemble approach. Inf Med Unlock. 2020;18:100277. https://doi.org/10.1016/j.imu.2019.100277.