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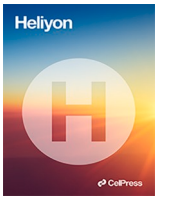

Research article

Decoding common genetic alterations between Barrett's esophagus and esophageal adenocarcinoma: A bioinformatics analysis

Pooya Jalali [a,1], Alireza Yaghoobi [a,1], Malihe Rezaee [a], Mohammad Reza Zabihi [b], Moein Piroozkhah [a], Shahram Aliyari [c], Zahra Salehi [d,e,*]

[a] Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c] Division of Applied Bioinformatics, German Cancer Research Center DKFZ Heidelberg, Iran
[d] Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
[e] Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Esophageal adenocarcinoma (EAC) is a common cancer with a poor prognosis in advanced stages. Therefore, early EAC diagnosis and treatment have gained attention in recent decades. It has been found that various pathological changes, particularly Barrett's Esophagus (BE), can occur in the esophageal tissue before the development of EAC. In this study, we aimed to identify the molecular contributor in BE to EAC progression by detecting the essential regulatory genes that are differentially expressed in both BE and EAC.

Materials and methods: We conducted a comprehensive bioinformatics analysis to detect BE and EAC-associated genes. The common differentially expressed genes (DEGs) and common single nucleotide polymorphisms (SNPs) were detected using the GEO and DisGeNET databases, respectively. Then, hub genes and the top modules within the protein-protein interaction network were identified. Moreover, the co-expression network of the top module by the HIPPIE database was constructed. Additionally, the gene regulatory network was constructed based on miRNAs and circRNAs. Lastly, we inspected the DGIdb database for possible interacted drugs.

Results: Our microarray dataset analysis identified 92 common DEGs between BE and EAC with significant enrichment in skin and epidermis development genes. The study also identified 22 common SNPs between BE and EAC. The top module of PPI network analysis included *SCEL, KRT6A, SPRR1A, SPRR1B, SPRR3, PPL, SPRR2B, EVPL,* and *CSTA*. We constructed a ceRNA network involving three specific mRNAs, 23 miRNAs, and 101 selected circRNAs. According to the results from the DGIdb database, TD101 was found to interact with the *KRT6A* gene.

Conclusion: The present study provides novel potential candidate genes that may be involved in the molecular association between Esophageal adenocarcinoma and Barrett's Esophagus, resulting in developing the diagnostic tools and therapeutic targets to prevent progression of BE to EAC.

* Corresponding author. Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Shariati Hospital, Karegar Ave, 14114, Tehran, Iran.
E-mail addresses: Zahra.salehi6463@yahoo.com, zsalehi@sina.tums.ac.ir (Z. Salehi).
[1] These authors contributed equally to this work.

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1. Introduction

Esophageal adenocarcinoma (EAC) and squamous cell carcinoma are two main subtypes of esophagus cancers. EAC is the predominant subtype in Western countries and has increased dramatically by six folds during the past three decades, more than any cancer

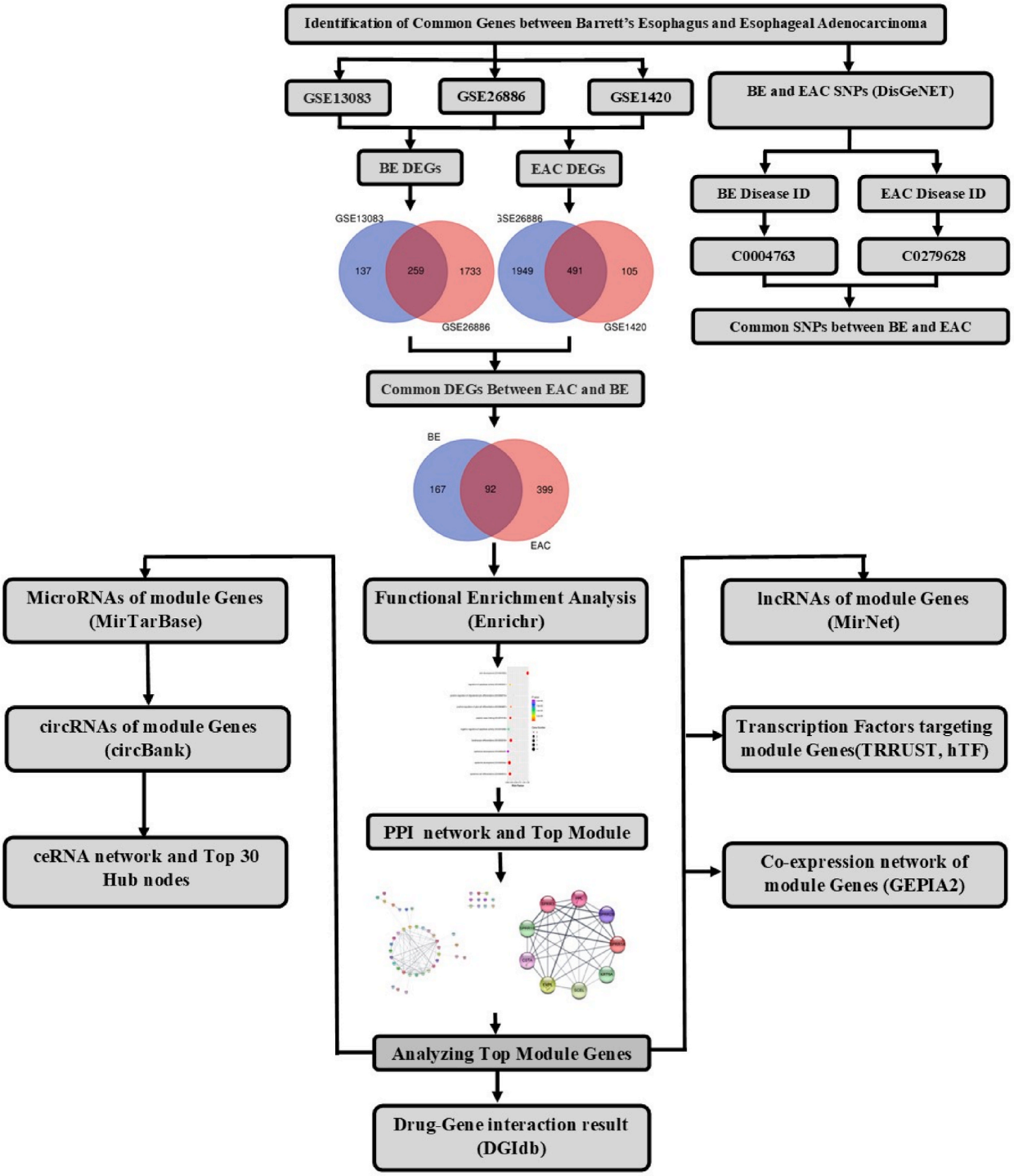

Fig. 1. The flowchart of the study. After analyzing three microarray datasets from the GEO database. we identified 92 differentially expressed genes (DEGs) that were commonly associated with Barrett's esophagus and esophageal adenocarcinoma (BE-EAC). We then employed various bioinformatics databases to conduct a comprehensive and systematic investigation of the functions of these DEGs in the progression of BE-EAC.

type [1,2]. EAC is responsible for two-thirds of esophageal cancer cases in high-income countries, with overweighting, gastric-esophageal reflux (GERD), and Barret's esophagus (BE) as its major contributors. Moreover, the decreasing infection rate of *H. pylori* in Western countries is inversely associated with increasing EAC in these countries. EAC has a high mortality rate and is the sixth cause of cancer death worldwide [3]. Despite the rapid developments in EAC conventional therapies, including endoscopic resection, surgery (esophagectomy), chemotherapy, and molecular targeted drugs (MDT), the 5-year survival rate is still below 20 % [4]. Currently, endoscopic surveillance is the most reliable screening strategy for diagnosing premalignant lesions of the esophagus caused by intestinal metaplasia of the distal esophagus, a condition known as Barrett's esophagus [5]. However, endoscopic surveillance is neither cost-effective for typical applications nor sensitive enough for detecting low-grade lesions; therefore, it is not commonly used for BE-EAC screening [6].

Barrett's esophagus is typically developed from gastric-esophageal reflux, a chronic condition in which acid, bile, and other stomach contents come up to the gastro-esophageal junction, inducing epithelial metaplasia after long-term exposure [6,7]. The primary low-grade metaplasia can further develop into higher grades and form dysplastic lesions, leading to malignancy [6]. Currently, BE is the only known precursor of EAC, and BE patients are 30–125 times more prone to EAC than healthy individuals [4,8]. This cascade of events from GERD to EAC is multifactorial and stems from a complex genetic, epigenetic, and environmental interaction [9]. Hence, it seems indispensable to focus on the genomic biomarkers and their role in cancer progression to develop novel diagnostic and therapeutic strategies capable of preventing or early intervening of BE-EAC.

Genome-wide association studies have identified several effective genes in the pathogenesis of BE to EAC progression. In a recent survey by Nan Yi et al. differentially expressed *MYO1A,* P2RY14*, RAB27A, ACE2, COL1A1, AADAC, ADRA2A,* and *LGALS4* genes were reported as potential diagnostic and prognostic biomarkers, with the *AADAC* and *ADRA2A* low-expression as the significant contributors to the EAC progression [1]. Moreover, it is demonstrated that obesity alone as a primary BE-EAC risk factor promotes *PPARG* overexpression through *ELF3, GATA6, KLF5,* and *EHF*, which are upstream of the fatty-acid synthesis signaling pathways. Overexpression of the *ELF3/GATA6/KLF5/EHF* and their downstream, *PPARG*, have been detected in both BE and EAC, indicating the potential role of these biomolecules as diagnostic biomarkers for early EAC intervention or prevention [10]. Like most cancers, EAC is associated with genomic alterations that stem from genomic instability at the early stages of the disease. Kumar et al. employed an integrated genomic approach to discover BE-EAC's genomic instability regulators. They revealed that *TPX2, TTK,* and *RAD54B* are upregulated in EAC patients and cell lines, and their suppression led to DNA damage inhibition [11]. Moreover, Dulak et al. demonstrated that *PIK3CA, ARID1A*, *SMAD4*, *SPG20*, *ELMO1, TLR4,* and DOCK2 significantly impacted BE to EAC progression [12].

In this study, we aimed to identify the common genes and SNPs involved in BE to EAC progression to introduce potentially actionable targets for either early EAC diagnosis or intervention. By analyzing the regulatory networks associated with these genes, we identified potential regulatory elements associated with the progression of BE to EAC, paving the way for the future development of pharmaceutical interventions and treatments that can precisely target the intricate molecular mechanisms involved in this process, thereby improving preventive care measures.

2. Materials and Methods

We conducted a comprehensive analysis of BE and EAC-associated genes obtained from GEO and DisGeNET databases using mixed bioinformatics techniques. First, we identified common differentially expressed genes (DEGs) using three microarray datasets obtained from the GEO database. Then, we used the DisGeNET database to identify the single nucleotide polymorphisms (SNPs) commonly associated with BE-EAC. To further investigate the function of GEO-extracted DEGs in BE-EAC, we established protein-protein interaction (PPI) networks and identified hub genes, as well as the top module within the PPI network. Moreover, we analyzed the co-expression network of the top module using the HIPPIE database. We also identified miRNAs targeting the common DEGs mRNAs using miRTarBase and ceRNAs sponging these miRNAs from the circBank database. Based on our findings, we constructed a ceRNA network including the selected mRNAs, miRNAs, and ceRNAs. Furthermore, we utilized the hTFtarget database to identify the key transcription factors (TFs) regulating the top module genes. Lastly, we inspected the DGIdb database for possible interacted drugs. The flowchart of this study is presented in Fig. 1.

2.1. Identification of common SNPs between BE and EAC using the DisGeNET database

For data collection, diseases' names were searched in the DisGeNET bioinformatics database [13], a widely available platform for collecting genomic data related to human disorders (https://www.disgenet.org). The DisGeNET IDs for Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) were extracted from the database, as shown in Table 1. As every disease is commonly referred to with different names, the information regarding each disease was collected from multiple sources, and duplicated genes were removed. Furthermore, the BE and EAC-related genes were extracted separately from the DisGeNET database, and through intersectional analysis, common DEGs between the two diseases were detected. The disgenet2r package was also employed to identify Single

Table 1
Barrett's esophagus and esophageal adenocarcinoma DisGeNET IDs.

No.	Disease name	Disease Abbreviation	DisGeNET IDs
1	Barrett Esophagus	BE	C0004763
2	Adenocarcinoma of Esophagus	EAC	C0279628

Nucleotide Polymorphisms (SNPs) correlated with the risk of BE and EAC, respectively [14,15]. Following, the common SNPs between BE and EAC were also obtained, and the common SNPs were then selected according to the DisGeNET cut-off score of >0.7. SNPs with no specific gene names were withdrawn from further analysis. Hence, two approaches were considered: (1) determination of the overlap between BE and EAC-related SNPs and the genes obtained from pairwise analysis of BE and EAC, and (2) checking whether the BE-associated SNPs were correlated with EAC-associated SNPs.

2.2. Microarray mining for BE and EAC

In this study, gene expression data from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo) were used to identify DEGs in BE-EAC. The inclusion criteria for the datasets were tissue samples of BE and EAC with a minimum sample size of 10 for each group, while the exclusion criteria were non-human studies, duplicated research, patients with prior radiotherapy or chemotherapy, and insufficient data. After a comprehensive analysis, GSE1420, GSE13083, and GSE26886 gene expression profiles were obtained from the GEO dataset (Table 2). Lastly, 8 EAC samples plus 8 HC (healthy control) samples from the GSE1420, 7 BE samples plus 7 HC samples from the GSE13083, and 20 BE samples, 21 EAC samples, and 19 HC samples from the GSE26886 fulfilled the inclusion criteria for further analysis. CEL files from Affymetrix microarrays were pre-processed using the Affy package (version 1.74.0; http://bioconductor.org/packages/release/bioc/html/affy.html) in R software (version 4.4.2; http://www.r-project.org/). The Robust Multi-array Average (RMA) method was used for the pre-processing, which included background correcting, normalizing, and calculating expression [16]. The latest annotation files were downloaded for re-annotation. To correct batch effects, The Limma package (version 3.52.2) [17] in R software and principal components analysis (PCA) were used. For DEGs analysis between BE and EAC two R packages ("GEOquery" and "limma") were employed. The threshold for the DEGs was established as adjusted-P value $<$ 0.05 and $|\log2 \text{ fold change (FC)}| \geq 1$. The Venn diagram was drawn using the jvenn tool (http://jvenn.toulouse.inra.fr/app/example.html), demonstrating the intersection between the two datasets.

2.3. Gene set enrichment analysis

In order to assess the functions and pathway enrichment of DEGs, we utilized the Gene Ontology (GO) database, which includes cellular components (CC), molecular functions (MF), and biological processes (BP). This analysis was conducted through the Enrichr platform (https://maayanlab.cloud/Enrichr/). Additionally, we analyzed the Kyoto Encyclopedia of Genes and Genomes (KEGG) through Enrichr to further evaluate DEG pathways [18]. The threshold was set as P-value <0.05 and $|\log FC| > 1$.

2.4. PPI network analysis, hub genes, and top module identification

Protein-protein interaction (PPI) network for common DEGs was created by STRING (https://string-db.org), and results were visualized by Cytoscape software (version 3.9.1; https://cytoscape.org). Additionally, the network's top module was identified using the MCODE plugin according to the following parameters: degree cutoff = 2, MCODE scores >5, node score cut-off = 0.2, max depth = 100, and k-score = 2. In this study, hub genes were defined as nodes with a degree >10 via the Centiscape plugin and were extracted.

2.5. Co-expression network

The co-expression network of top module genes was retrieved using HIPPIE (http://cbdm-01.zdv.uni-mainz.de/~mschaefer/hippie/) [19]. We specifically focused on the co-expression network in esophageal tissues, considering the HIPPIE confidence score of 0.01 and a high confidence level greater than 0.73. To construct the network, we employed Cytoscape software (version 3.9.1; https://cytoscape.org).

2.6. microRNAs/LncRNAs/circRNAs-mRNA network

In this study, miRTarBase (https://mirtarbase.cuhk.edu.cn), mirNET (https://www.mirnet.ca/), and circBank databases (http://www.circbank.cn) were employed to identify miRNAs, long non-coding RNAs (lncRNAs), and circRNAs respectively. Briefly, to

Table 2
Characteristics of the studied microarray dataset.

No	GSE no.	GPL/Platform	No. of samples			Sample type	Update (year)	Country	Total
			BE (n)	EAC (n)	HC (n)				
1	GSE26886	Affymetrix Human Genome U133 Plus 2.0 Array	20	21	19	Tissue	2019	Germany	60
2	GSE13083	Affymetrix Human Genome U133A Array	7	_	7	Tissue	2008	USA	14
3	GSE1420	Affymetrix Human Genome U133A Array	_	8	8	Tissue	2004	USA	16

BE, Barret's Esophagus; EAC, Esophageal Adenocarcinoma HC: Healthy Control.

obtain the top module gene regulating network, we first extracted miRNA data from the miRTarBase database. Then, we used miRNA data to obtain a ceRNA network of mRNA-miRNA-circRNA connections based on miRTarBase and circBank databases. CircRNAs exceeding a total score of 1000 were selected as the threshold and subsequently subjected to additional analysis. Competitive endogenous RNA (ceRNA) network was constructed by Cytoscape software 3.9.1 (https://cytoscape.org) [20], and hub nodes were identified by the cytoHubba package [21] based on the degree method. Moreover, by the use of experimentally validated miRNAs obtained from the miRTarBase, we identified a list of lncRNAs related to top module genes gene from the mirNET database, a miRNA-centric network visual analytics platform (https://www.mirnet.ca/). For each experimentally validated miRNA obtained from miRTarBase, we excreted the related lncRNAs and analyzed their overlap by utilizing the Genomics web tool (https://bioinformatics.psb.ugent.be/webtools/Venn/).

2.7. Transcription factor interactions

In order to gain insight into the regulation of genes through transcription factors, we extracted the transcription factors regulating these target genes and ran an analysis on their interaction regulations by the hTFtarget database (https://bio.tools/hTFtarget) [22].

2.8. DIGIdb

In this study, we evaluated the interactions between drugs and the retrieved DEGs using the Drug Gene Interaction Database (DGIdb) (https://www.dgidb.org/), a web-based platform containing information on drug-gene interactions from publications, databases, and other web-based sources. Finally, we prepared a list of possible therapeutic medicines according to the drugs approved by the Food and Drug Administration (FDA) in the DrugBank database (30).

3. Results

3.1. Identification of common SNPs between BE and EAC

By analyzing data from the DisGeNet database, a total of 478 genes involved in BE and 468 genes involved in EAC were identified (Supplementary file 1). Upon conducting further analysis, we established that 236 of these genes were common to both BE and EAC. Moreover, the study identified 31 and 33 SNPs associated with BE and EAC, respectively, each with a DisGeNET score exceeding 0.7. These SNPs were found to be linked to 18 and 20 genes related to BE and EAC, respectively. The study also identified 22 common SNPs between BE and EAC (Table 3).

Table 3
Characteristics of common SNPs between Barrett's Esophagus and Esophageal Adenocarcinoma.

Variant ID	Gene	Chromosome	Position	Consequence
rs10419226	*CRTC1*	19	18692362	intron variant
rs11789015	*BARX1*	9	93953746	intron variant
rs2687201	*FOXP1*	6	61285855	intergenic variant
rs17451754	*LOC107985847*	3	70879779	intergenic variant
rs3784262	*CFTR*	2	7377100	intergenic variant
rs10108511	*ALDH1A2*	7	117616658	intron variant
rs11901649	*LINC00208*	15	57960908	intron variant
rs1247942	*APOB*	3	184065565	downstream gene variant
rs17749155	*MSRA*	8	11578007	non-coding transcript exon variant
rs1979654	*ASZ1;CFTR*	2	21027351	intron variant
rs199620551	*LOC283665* *ALDH1A2*	2	150929228	intergenic variant
rs2178146	*LOC105375146*	12	114235918	downstream gene variant
rs2464469	*MIR329-2* *MIR323A* *MIR329-1* *MIR758* *MEG8* *MIR1197*	X	140863896	intergenic variant
rs2687202	*GDF7*	8	10210563	intron variant
rs4676893	*TPPP*	16	86363229	TF binding site variant
rs4800353	*KHDRBS2*	19	18693485	intron variant
rs62423175	*LOC107986152*	16	86430089	downstream gene variant
rs7255	*TMOD1*	7	117400063	intron variant
rs7632500	*OR12D3* *OR5V1*	5	58270246	intergenic variant
rs7852462	*CEP72* *TPPP*	15	58069827	intron variant
rs9257809	*MHC*	3	70880832	regulatory region variant
rs9918259	*TPPP*	7	9966714	intron variant

3.2. Common genetic alterations between BE and EAC

Microarray data from the GEO database was downloaded to obtain BE and EAC-associated DEGs. Based on our previously described inclusion criteria, BE, EAC, and normal tissue gene expression profiles of GSE26886, GSE1420, and GSE13083 were included in the analysis. Using adjusted *P*-value <0.05 and $|\log FC| > 1$ as the cut-off criterion, 491 EAC-related DEGs (252 upregulated and 239 downregulated) and 259 BE-related DEGs (150 down regulated and 159 upregulated) were identified, respectively (Fig. 2A and B and Supplementary File 1). After applying integrated bioinformatics analysis, 92 common DEGs, including 74 down regulated and 18 upregulated DEGs between BE and EAC were detected (Supplementary File 1) (Fig. 2C).

Furthermore, we employed pairwise analysis to determine the genes overlapping between the DisGeNET database and microarray datasets. The results demonstrated 23-BE (Fig. 2D) and 49-EAC (Fig. 2E) associated genes that were common to both the DisGeNET database and microarray datasets (Supplementary File 1).

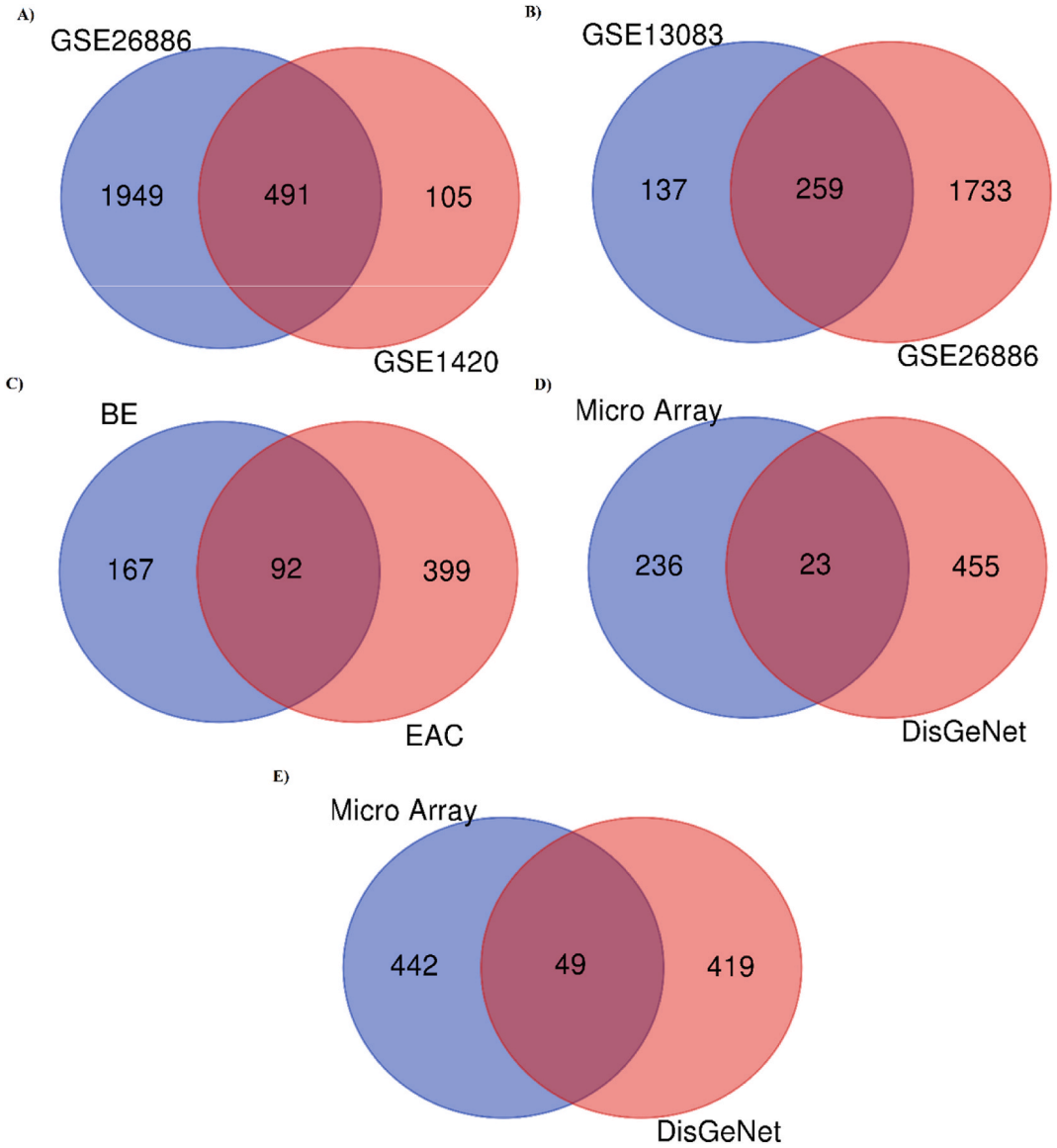

Fig. 2. Venn diagram for obtaining shared differentially expressed genes (DEGs). (A) Common differentially expressed genes (DEGs) between esophageal adenocarcinoma (EAC) datasets. GSE26886 and GSE 1420 were investigated for establishing EAC-associated DEGs based on previously described inclusion criteria. Subsequently, 491 common DEGs were achieved between the two studies. (B) Common DEGs between Barrett's esophagus (BE) datasets. As evident, 259 common DEGs were identified between the two included studies for BE. (C) Common DEGs between EAC and BE were acquired by the pairwise analysis of the resulting common DEGs for BE and EAC. (D) Common DEGs between Micro Array and DisGeNet BE DEGs. (E) Common DEGs between Micro Array and DisGeNet EAC genes.

3.3. Functional enrichment analysis

GO and KEGG pathway enrichment analyses evaluated the potential biological function of 92 common DEGs with statistical significance of P values ≤ 0.05 (Supplementary File 1). The selected genes were mainly enriched in skin development (GO: 0043588; BP), epidermis development (GO: 0008544; BP), keratinocyte differentiation (GO: 0030216; BP) (Fig. 3A), protease binding (GO: 0002020; MF), phospholipase inhibitor activity (GO: 0004859; MF), endopeptidase inhibitor activity (GO: 0004866; MF) (Fig. 3B), and cornified envelope (GO: 0001533; CC), intermediate filament (GO: 0005882; CC), intermediate filament cytoskeleton (GO: 0045111; CC) (Fig. 3C). The GO results are consistent with those of the KEGG pathway analysis. KEGG pathway analysis showed insulin signaling and estrogen signaling pathways are mostly related to common DEGs (Fig. 3D and Supplementary File 1).

3.4. PPI and hub genes identification

The PPI network of 92 overlapped genes was created with 89 nodes and 100 edges by STRING. The degree cut-off was set >10 using the Centiscape plugin, Cytoscape software, and the top 5 ranking genes, including Sciellin (*SCEL*), Keratin 6A (*KRT6A*), Small Proline Rich Protein 1A (*SPRR1A*), Small Proline Rich Protein 1B (*SPRR1B*), Small Proline Rich Protein 3 (*SPRR3*) were detected as hub genes

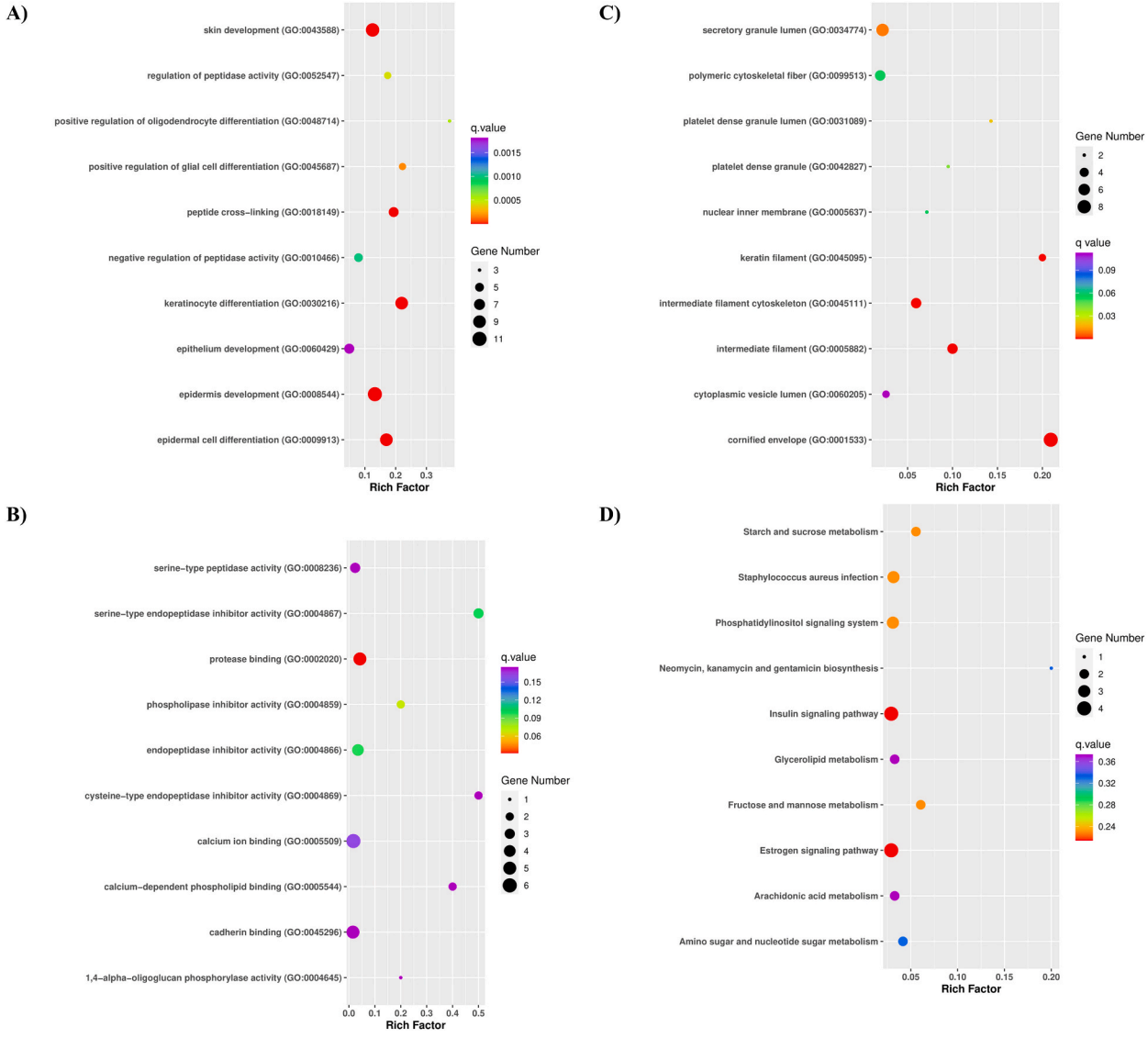

Fig. 3. Gene set enrichment analysis. (A) Biological process (BP) in BE and EAC mainly shows epidermal cell growth and differentiation as the dominant process associated with BE-EAC common DEGs. (B) Molecular function (MF) enrichment analysis revealed the dominance of the endopeptidase inhibition activity in BE and EAC common DEGs. (C) Cellular component (CC) analysis revealed that cytoskeletal filament structure is commonly involved in BE-EAC DEGs. (D) KEGG pathway analysis based on rich factor and p-value in BE-EAC associated DEGs.

(Fig. 4A). Also, two modules were identified which one of them had score >5. There were 9 nodes and 31 edges in the identified top module, including *PPL, SPRR2B, SPRR1A, KRT6A, SCEL, EVPL, CSTA, SPRR1B*, and *SPRR3* (Fig. 4B).

3.5. Gene regulatory network inference

After establishing the most significant module based on the PPI network, we identified the 23 potential miRNAs involved in regulating the *PPL, SPRR3*, and *SPRR1A* using the mirTarbase database. No miRNAs were identified targeting *SPRR2B, KRT6A, SCEL, EVPL, CSTA,* and *SPRR1B*. Furthermore, over 180000 circRNAs were detected using the circBank database, associated with the 23 potential miRNAs. After considering the Tot score cut-off >1000, only 101 circRNA remained to construct the ceRNA network. The ceRNA was subsequently constructed with 3 mRNAs, 23 miRNAs, and 101 circRNAs (Fig. 5A). Then, 30 hub nodes identified by cytoHubba based on the degree, including *PPL* (score: 22), *hsa-miR-1304-3p* (score: 42), *hsa-miR-6807-3p* (score: 27), *hsa-miR-153-5p* (score: 13), *hsa-miR-627-3p* (score: 10) (Fig. 5B).

In addition, we identified 243 lncRNAs, which were related to 9 miRNAs among 23 detected miRNAs, using the information from the miRNet database. We further performed an analysis to detect the common lncRNAs between those related to each miRNA, and as a result, only *lncRNA OIP5-AS1*, also known by the alias Cyrano, was found. It has been revealed that *OIP5-AS1* was involved in the regulation of developmental and cellular processes, including proliferation, apoptosis, and mitosis [23].

Further, we investigated the possible transcription factors using the hTFtarget database. We found 458 TFs for *PPL* gene, 32 TFs for *SPRR2B* gene, 25 TFs for *SPRR1A* gene, 100 TFs for *KRT6A* gene, 109 TFs for *SCEL* gene, 348 TFs for *EVPL* gene, 103 TFs for *CSTA* gene, 71 TFs for *SPRR1B*, and 41 TFs for SPRR3, which may be involved in regulating the expression of these genes (Fig. 6 and Supplementary File 1). Further analysis showed that 5 TFs, including *FOS, JUND, SPI1, GRHL2,* and *FOXA1* were common among the candidate genes.

3.6. Co-expression network of the overlapped genes

The co-expression network was analyzed through the HIPPIE database. The analysis revealed the presence of 6 main subnetworks, comprising 74 nodes significantly correlated with the top module DEGs, as shown in Fig. 7. In addition, several overlapped nodes, including *CUL1, DSP, ITGA9, CUL2, WTAP, METLL3,* and *GOLGA2*, linked these subnetworks (Fig. 7). Specifically, *CUL1* was associated with *PPL-KRT6A, DSP with EVPL-CSTA*, and *ITGA9* with *EVPL-SPRR1B*. Additionally, *CUL2, WTAP*, and *METLL3* were linked with *CSTA-KRT6A*, while *GOLGA2* was linked with *KRT6A-SCEL*. Notably, the associations between these genes were particularly strong for *CUL2* and *DSP*. Cullin-2 (*CUL2*), which belongs to the Cullin family, acts as a scaffold protein for Elongin B and C, Rbx1, and multiple substrate recognition receptors to establish E3 ubiquitin ligases [24,25]. Cullin-RING E3 ubiquitin ligase complexes are central in directing cellular proteins for ubiquitination-dependent protein degradation through the 26S proteasome [24,25]. The *DSP* gene is responsible for encoding the protein desmoplakin, which is crucial in anchoring intermediate filaments to desmosomal plaques and is an essential component of functional desmosomes. Additionally, desmosomes are believed to be involved in other crucial

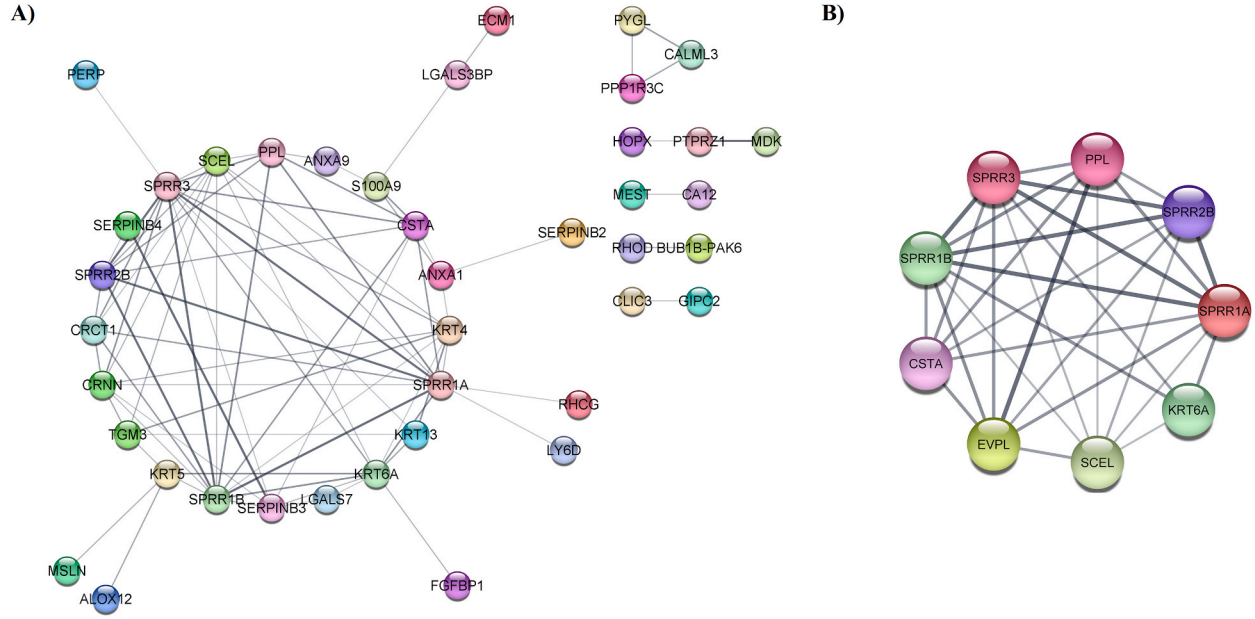

Fig. 4. The Protein-protein interaction network and top module of differentially expressed genes (DEGs)**.** (A) Protein-protein interaction network of the 92 common DEGs between Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC). (B) The most significant module of DEGs, containing 5 hub genes, is shown with their interaction.

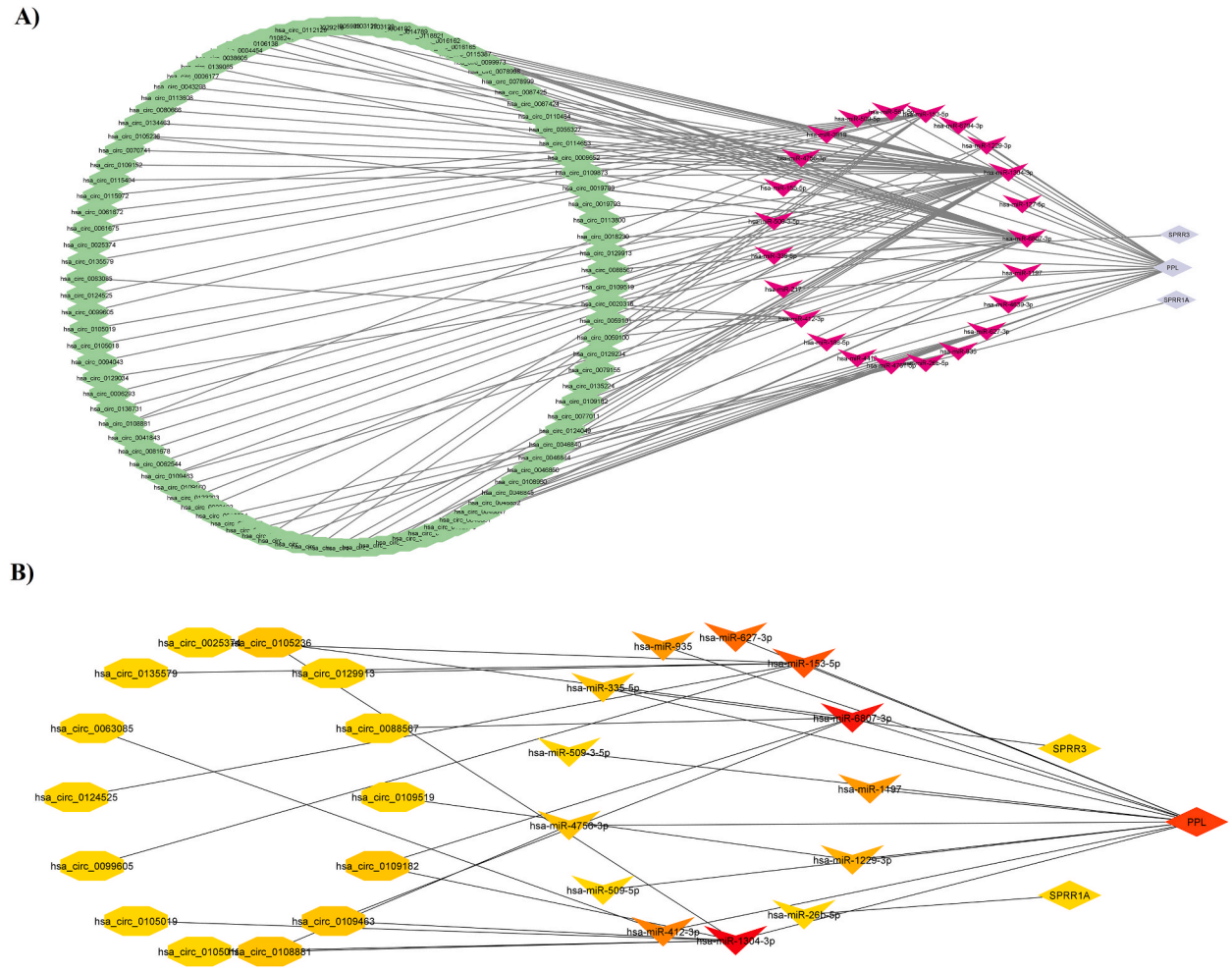

Fig. 5. The ceRNA network and hub nodes. (A) The ceRNA network was constructed based on the most significant module of the PPI network and by 3 mRNAs, 23 miRNAs, and 101 selected circRNAs. (B) The top 30 hub nodes were identified by cytoHubba based on the degree method; yellow to red refers to the increment of degree value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cellular functions, including chemical signaling pathways, cellular differentiation, and apoptosis [26].

3.7. Drug-gene interaction

According to the results from the DGIdb database, only one drug named TD101 was predicted to have a potential therapeutic effect on BE-EAC patients. This drug interacts with KRT6A and has already been shown to be effective in relieving symptoms of Pachyonychia Congenita, a rare condition related to mutations in keratin-encoding genes.

4. Discussion

Esophageal adenocarcinoma (EAC) is a type of cancer with a relatively poor prognosis, associated with a high rate of relapse, metastasis, and mortality. The incidence of EAC has been steadily increasing and is expected to continue to rise rapidly in the coming years [27]. EAC is often diagnosed at an advanced stage, making it more challenging to treat and resulting in a lower prognosis. On the other hand, despite the advances in EAC treatment, it remains prone to the development of resistance before and after treatment, resulting in a poor prognosis [28]. Therefore, developing novel potential biomarkers for early diagnosis or treatment of EAC using biology technology has gained growing attention in recent decades. In this line, various pathological changes can occur in the esophageal tissue before the development of EAC. Identifying these conditions enables us to make early diagnosis and apply multiple therapeutic approaches to prevent cancer formation. Barrett's Esophagus (BE), a premalignant change in the esophageal lining, is typically the precursor to EAC [29]. However, it has been established that EAC and BE are multifactorial, and the precise molecular correlation between EAC and BE has remained unknown. Therefore, the present study is designed to explore common DEGs between

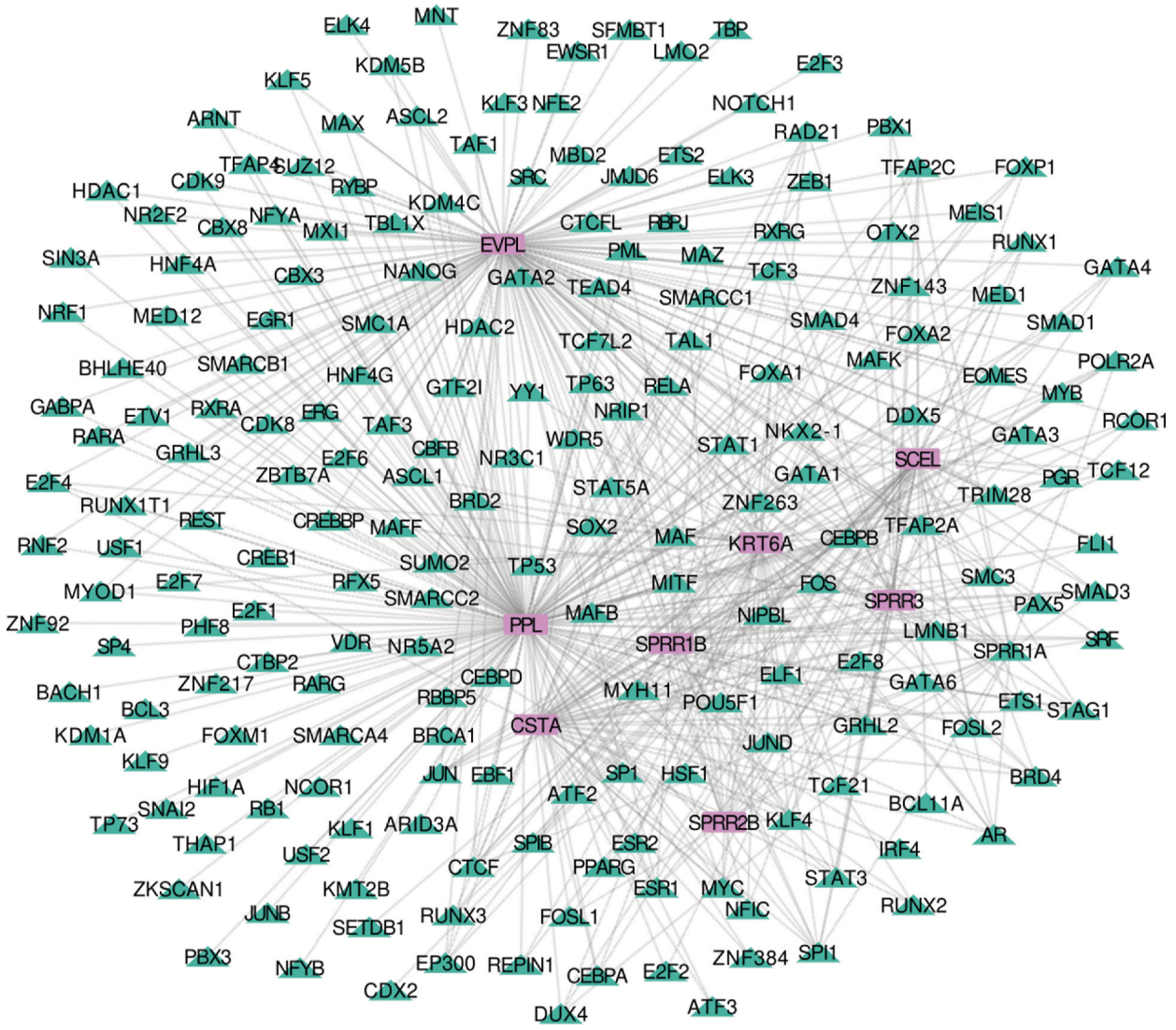

Fig. 6. The TFs of module genes. Five TFs, including FOS, JUND, SPI1, GRHL2, and FOXA1 were common among the candidate genes.

EAC and BE and their various roles to provide potential diagnostic factors and therapeutic targets.

In the present study, we analyzed transcriptomic data of human EAC and BE using publicly available datasets to identify common DEGs in these two conditions. We found 92 common DEGs between BE and EAC, including 78 up-regulated and 14 down-regulated genes. Previously, a similar study evaluated the EAC- and BE-associated microarray datasets and found 403 DEGs, consisting of 236 upregulated and 167 downregulated genes involved in the cell cycle and replication pathways. Also, they identified a high correlation between the DEGs and gender. Also, five common hub gene signatures, namely, *PRPF4*, *SRSF1*, *HNRNPM*, *DHX9*, and *ORC2*, were identified between BE and EAC. These five genes were found to mostly enrich RNA metabolism and splicosomes, and play a key role in esophageal cancer development and progress [30]. In this line, another recent investigation analyzed the common DEGs between BE and EAC using microarray analysis that showed 27 up- and 104 down-regulated genes with high involvement in tumorigenesis. Their further analysis revealed 5 up-regulated genes (*MYO1A, ACE2, COL1A1, LGALS4*, and *ADRA2A*) and 3 down-regulated genes (*AADAC, RAB27A,* and P2RY14) could consider potential genes related to BE-EAC or contribute to EAC pathogenesis and progression [31]. Alongside genes, the SNPs are also important in disease development and progression. For instance, a genome-wide association study identified three associations between BE and EAC including, at 19p13 (rs10419226) in the *CRTC1* gene, at 9q22 (rs11789015) in the *BARX1* gene, and 3p14 (rs2687201) near the *FOXP1* gene [32]. In this regard, we found 22 common SNPs between BE and EAC.

The common DEGs between BE and EAC investigated in this study were primarily enriched in skin and epidermis development, keratinocyte differentiation, development, and proliferation. Keratinocytes are the primary cell type in the epidermis and play a crucial role in skin repair by migrating, proliferating, and differentiating to restore the epidermal barrier [33,34]. Pathologically, BE is

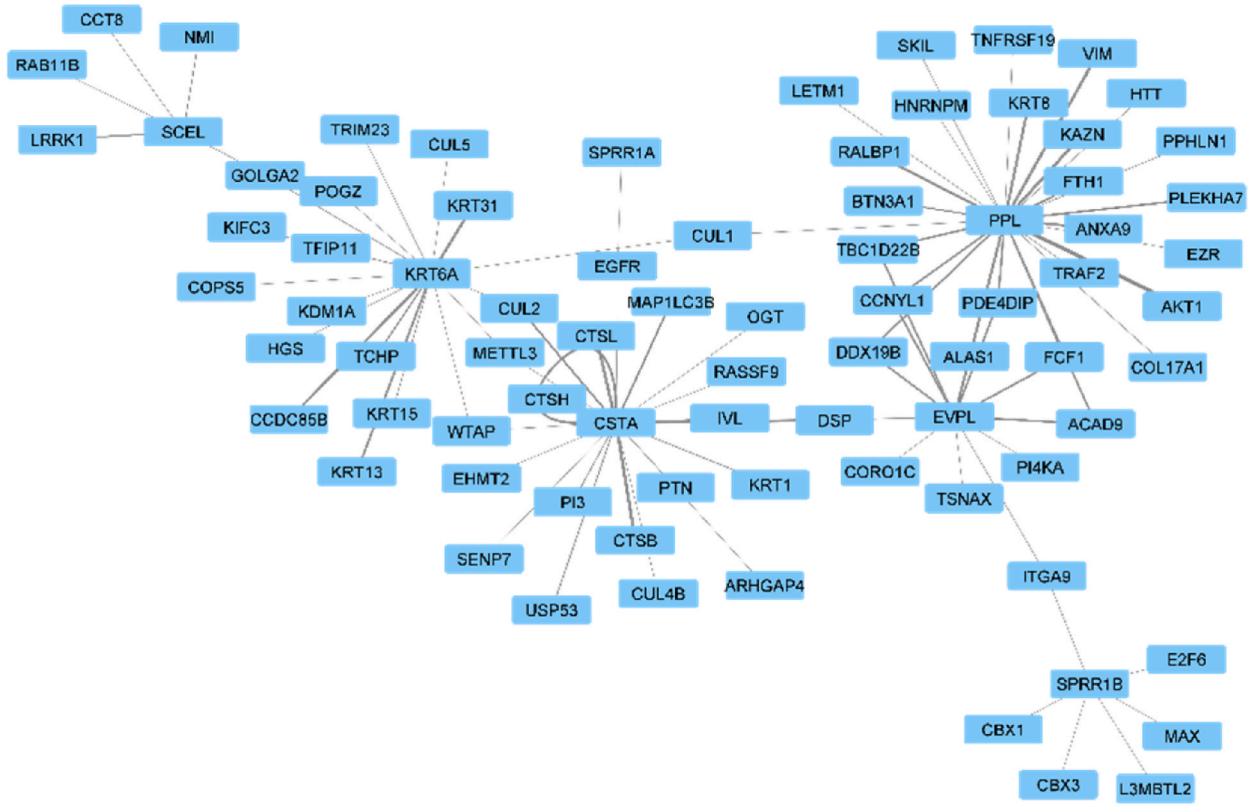

Fig. 7. Tissue-specific co-expression network of the top module genes. The edges with more thickness and dark colors display higher confidence. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

characterized by the phenotypic shift of esophageal epidermal cells toward intestinal differentiation. Several molecular changes in the basal esophageal keratinocytes, such as ectopic expression of CDX2 and activation of CDX2-related signaling pathways, were identified to be associated with BE and EAC development [35,36]. Therefore, it could be implied that impairment of keratinocyte-related functions contributed to both EAC and BE. Additionally, our KEGG pathway analysis revealed that the common DEGs are mainly involved in insulin and estrogen signaling pathways. Previous studies reported that insulin/insulin-like growth factor 1 and estrogen receptor (ER) signaling cascades, which highly interact with each other, are two of the most relevant metabolic pathways involved in the cocarcinogenic processes, importantly esophageal cancers [37,38] as well as BE development and progression to cancer formation [39,40].

It has been revealed that several SNPs are involved in both BE and EAC [41]. For instance, a recent study identified a potential genetic variant called rs10423674, which may contribute to functional risks in both BE and EAC. Additionally, a risk-enhancing element was found on the chromosome 19p13.11 region involved in BE and EAC. This discovery was made through luciferase reporter enhancer activity assays, CRISPR genome editing techniques, and gene expression profiling. To further bolster the identification of potential target genes in this specific location, the study integrated publicly available genotype-expression correlation from normal human tissues and performed statistical analyses to assess colocalization [42]. Similarly, using expression quantitative trait loci (eQTL) data, three BE/ECA-associated genetic variants were reported, including variant rs7754014 on 6q25 represents an eQTL for the gene *SLC22A3* in esophageal mucosa, variant rs147462972 on 5p15 defines an eQTL for the gene *SLC9A3* in esophageal mucosa, and variant rs1540 on 16q23 regulates the *CFDP1* expression in gastroesophageal junction [43]. Importantly, *SLC9A3* overexpression has already been found to be associated with gastroesophageal reflux, which is a contributing factor to the risk of BE and EAC [44].

Further PPI analysis identified the top 5 ranking genes, including *SCEL*, *KRT6A*, *SPRR1A*, *SPRR1B*, and *SPRR3*, as hub genes. In addition to these 5 genes, 4 other genes were involved in the top module network, including *PPL*, *SPRR2B*, *EVPL*, and *CSTA*. We also identified the possible transcription factors for these genes and found that FOS, JUND, SPI1, GRHL2, and FOXA1 were common among them. Previous studies have shown that *KRT6A*, *PPL*, *EVPL,* and *CSTA* genes, which are expressed in stratified squamous epithelia, have been identified as a potential biomarker for esophageal squamous cell cancer (ESCC) [45–47]. *SPRR* genes play a crucial role in epithelial cell differentiation and structure [48], and *EVPL* and *SCEL* are cornified envelope genes [49–51]. However, it is worth noting that the lack of further evidence regarding the role of these genes in EAC and BE does not diminish the potential significance of their function within the epithelial cells, which could potentially elucidate crucial aspects of the pathophysiology of BE and EAC. Notably, *KRT6A* was found to be downregulated in human Barrett's esophagus tissue samples compared to normal esophagus tissue samples [52]. Also, our drug-gene interaction analysis introduced TD101, which interacts with *KRT6A*, to potentially have a therapeutic effect

on BE-EAC patients. So far, the TD101, a small interfering RNA (siRNA), was examined for treating pachyonychia congenita and demonstrated to be efficacious and safe in the phase Ib study (NCT00716014). However, there were no clinical trials regarding the effects of this drug in EAC or BE.

Over the past decade, non-coding RNAs (ncRNAs) have emerged as crucial regulatory factors in various biological processes. Although no proteins are encoded by ncRNAs, these molecules regulate gene expression at the transcriptional, post-transcriptional, translational, and epigenetic levels, as well as mRNA stability. They can be broadly classified into two main categories based on their size: small ncRNAs (<200 nucleotides) and long ncRNAs (>200 nucleotides). Small ncRNAs include microRNAs (miRNAs) and circular RNAs (circRNAs). They have been identified to be involved in numerous diseases, including cancer, cardiovascular diseases, and neurological disorders [53]. In addition, miRNA can interact with both circRNA and lncRNA through various mechanisms, including (1) miRNAs can bind to circRNA molecules via complementary base-pairing, leading to the regulation of circRNA expression and function [54], (2) circRNAs and lncRNAs can function as miRNA sponges and compete with mRNAs for miRNA binding, thereby affect the miRNA availability [54,55], (3) lncRNAs can act as molecular scaffolds, guides, or decoys for miRNAs which can modify the interaction between miRNAs and their target mRNAs [55–57]. In this regard, a growing body of studies reported that dysregulation of ncRNAs contributed to the molecular mechanisms driving the development and progression of both BE and EAC [58]. For instance, ncRNAs regulate the epithelial-to-mesenchymal transition (EMT) process, which plays an essential role in the progression of BE to EAC [59–61]. In the present study, we found 23 potential miRNAs involved in regulating the genes of the top module and over 180000 circRNAs, which were associated with the detected miRNAs. Moreover, 101 circRNAs were detected with the most significant correlation based on the determined criteria. Also, we identified 243 lncRNAs related to the detected miRNAs, and *lncRNA OIP5-AS1* was the shared one between them. The role of *lncRNA OIP5-AS1*, encoded by the anti-sense of the *OIP5* gene, in promoting the EMT process, migration, proliferation, and invasion of tumor cells was elucidated in diverse cancers [62,63]. Consistently, recent investigations illustrated that *lncRNA OIP5-AS1* could enhance the proliferation, migration, and invasion of esophageal cancer cells by sponging *miR-30a* [64,65].

Moreover, ncRNAs exert their interplay within competing endogenous RNA (ceRNA) networks, which exploit the crosstalk between different ncRNAs and mRNAs through their shared microRNA binding sites. Consequently, they competitively interact and subsequently affect each other's expression at the post-transcription level. Dysregulation of the interactions within ceRNA network can lead to miRNA-mediated loss of regulation and play a pivotal role in disease progression, carcinogenesis, and metastasis [66]. In this way, we constructed the ceRNA network with the specific 3 mRNAs, 23 miRNAs, and 101 circRNAs. The hub node with the highest score in this ceRNA network was *hsa-miR-1304-3p*. Consistently, a qRT-PCR analysis of the tissues and serum of patients with esophageal carcinoma showed the increased expression of *miR-1304* [67]. Also, *miR-1304* upregulation, particularly *miR-1304-p*, was found to be related to the advanced stages of esophageal carcinoma. These findings indicated its potential diagnostic value in identifying esophageal carcinoma, tumor size, differentiation, and stage. Further protein co-expression analysis revealed that co-expression of *miR-1304* with the *EGF* (epidermal growth factor) gene was the most frequent [67]. Conclusively, the complex regulatory role of ncRNAs and ceRNA network enhances the comprehension of gene expression and its dysregulation in BE and EAC. Investigating the roles of ncRNAs and interpreting the interactions within ceRNA networks could provide potential insight into the development of novel therapeutic targets and biomarkers.

The co-expression network of the top module genes consisted of 74 genes and 6 main subnetworks. In addition, several overlapped genes, including *CUL1, DSP, ITGA9, CUL2, WTAP, METLL3*, and *GOLGA2* linked these subnetworks. Previously, it has been shown that the *CUL1* and *CUL2* genes encode Cullin-1, a protein that is part of the SCF (Skp1-Cullin-1-F-box protein) complex and the protein Cullin-2, which is a component of the Cullin-RING E3 ligase (CRL2) complex, respectively [68–71]. These complexes regulate protein degradation through the ubiquitin-proteasome system and play a crucial role in regulating various cellular processes such as cell cycle progression, DNA repair, and signal transduction. Dysregulation of these two genes could contribute to tumorigenesis [69,72,73]. The *DSP* gene encodes desmoplakin protein, which plays a crucial role in the structure and function of desmosomes to connect intermediate filaments in epithelial cells. Dysregulation of *DSP* was identified to increase cell proliferation and participate in carcinogenesis [74]. The *ITGA9* gene exerts an important function in cell adhesion, proliferation, and migration, with its dysregulation correlated to carcinogenesis and cancer progression [75,76]. The *WTAP* and *METLL3* genes have been shown to associate with the N6-methyladenosine (m6A) methyltransferase complex in regulating RNA stability, which plays a crucial role in regulating various biological processes, including mRNA stability, splicing, localization, and translation. The *WTAP* and *METLL3* genes have been implicated in accelerating tumor progression and metastasis [77–79]. Also, a recent study demonstrated the role of the *METLL3* gene in the modification of the EMT process [77]. Dysregulation of the *GOLGA2* gene, encoding protein Golgin A2 with critical roles in Golgi organization, protein trafficking, and cellular homeostasis, has been found to be related to cancer pathogenesis [80].

In this study, we provided a comprehensive bioinformatics analysis to identify the genetic and epigenetic correlation between BE and EAC with their function. However, this study has been limited by the lack of experimental data to further confirm these results, and for future research, conducting functional experiments to validate these computational findings is necessary. Furthermore, while the GEO database represents an invaluable repository, it amalgamates data from a variety of origins and technological platforms, which may inherently introduce biases and inconsistencies. As such, it is imperative to approach the interpretation of these correlations and their subsequent implications with a degree of caution. Our study also explored the regulatory roles of circRNAs, miRNAs, and lncRNAs in gene expression. Although this investigation provides a glimpse into possible regulatory mechanisms, it's important to recognize that our findings are based on computational models. Experimental validation is essential to confirm these relationships and ascertain a definitive cause-and-effect linkage. In this regard, by using experimentally validated microRNAs for constructing ceRNA network and determining the regulatory elements of the top module genes, we tried to partially compensate for this limitation of our study. Furthermore, it is worth noting that in silico and bioinformatics approaches have proven to be valuable in gaining insights into

complex biological processes and have led to significant discoveries in the field of genomics and cancer research [81,82].

5. Conclusion

In conclusion, we identified 92 common DEGs between BE and EAC, with significant enrichment in genes involved in skin and epidermis development. The *SCEL, KRT6A, SPRR1A, SPRR1B, SPRR3, PPL, SPRR2B, EVPL*, and *CSTA* genes were involved in the top module. Furthermore, we identified the potential microRNAs, circRNAs, and lncRNAs and consequently constructed the ceRNA network that may be involved in regulating the genes of the top module. The co-expression network of these genes also was constructed that 7 overlapped genes, including *CUL1, DSP, ITGA9, CUL2, WTAP, METLL3*, and *GOLGA2*, linked these subnetworks Furthermore, our analysis of drug-gene interactions predicted that a drug called TD101, which interacts with the *KRT6A* gene, may have a potential therapeutic effect on BE-EAC patients. Although further validation is required, our study provides valuable insights into potential candidate genes that may be involved in the molecular association between BE and EAC. This could pave the way for developing diagnostic tools and therapeutic targets for these conditions.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The results of the computer-based analyses conducted in this study, as well as the datasets presented, can be found in online databases. The article includes the names of these databases and their respective identification numbers.

CRediT authorship contribution statement

Pooya Jalali: Project administration, Methodology, Formal analysis, Conceptualization. **Alireza Yaghoobi:** Writing – original draft, Investigation. **Malihe Rezaee:** Writing – original draft. **Mohammad Reza Zabihi:** Formal analysis, Data curation. **Moein Piroozkhah:** Formal analysis, Data curation. **Shahram Aliyari:** Formal analysis, Data curation. **Zahra Salehi:** Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

BE	Barrpet's Esophagus
BP	Biological Process
CC	Cellular Component
Ce-RNA	Competing endogenous RNA
CRL2	Cullin-RING E3 ligase
DEG	Differential Expressed Gene
EAC	Esophagus Adenocarcinoma
EGF	Epidermal Growth Factor
ER	Estrogen Receptor
EMT	Epithelial-to-Mesenchymal Transition
eQTL	Expression Quantitative Trait Loci
ESCC	Esophageal Squamous Cell Cancer
FC	Fold Change
GERD	Gastrointestinal Reflux Disease

GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
KRT6A	Keratin 6A
lncRNA	Long non-coding RNAs
MDT	Molecular Targeted Drug
MF	Molecular Function
miRNAs	MicroRNAs
m6A	N6-methyladenosine
PPI	Protein-Protein Interaction
RMA	Robust Multi-array Average
SCEL	Sciellin
SCF	Skp1-Cullin-1-F-box protein
siRNA	small interfering RNA
SNP	Single Nucleotide Polymorphisms
SPRR1A	Small Proline Rich Protein 1A
SPRR1B	Small Proline Rich Protein 1B
SPRR3	Small Proline Rich Protein 3
TF	Transcription Factor

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31194.

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