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# Molecular mechanism detection of stage I to stage II transition of esophageal squamous cell carcinoma: a system biology approach

**Mitra Rezaei[1], Fatemeh Bandarian[2], Farideh Razi[3], Zahra Razzaghi[4], Ayad Bahadori Monfared[5], Babak Arjmand[6,7], Reza M Robati[8], Alireza Ahmadzadeh[9,10]**

*[1]Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*[2]Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
*[3]Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
*[4]Laser application in medical sciences research center, Shahid Beheshti University of medical sciences, Tehran, Iran*
*[5]Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*[6]Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*
*[7]Iranian Cancer Control Center (MACSA), Tehran, Iran*
*[8]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*[9]Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
*[10]Department of lab sciences, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

## ABSTRACT

**Aim**: Molecular mechanism detection of stage I to stage II transition of esophageal squamous cell carcinoma via protein-protein interaction (PPI) network analysis is the main aim of this study.

**Background**: Esophageal cancer (EC) is recognized as cancer with a very poor prognosis and malignancy. It is characterized by a high prevalence rate within the world and a very low survival rate, even with treatment.

**Methods**: To detect esophageal squamous cell carcinoma (ESCC) related genes, gene expression profiles (GEPs) of GSE161533 from the Gene Expression Omnibus (GEO) database were considered to be analyzed. Data was evaluated via the GEO2R program to explore the significant differential genes (DEGs) associated to stages I and II of esophageal squamous cell carcinoma. Each analysis's top 250 significant DEGs were evaluated, and the non-common genes were assessed via PPI network analysis. The hub-bottleneck DEGs were determined and enriched via gene ontology.

**Results:** Results indicate 373 significant DEGs discriminate stage I from stage II. PPI network analysis associated with gene expression assessment showed that COL1A1, SERPINE1, PDGFRB, AURKA, TGFBI, LGALS3, BRCA1, and TFRC are the critical DEGs which are related to ESCC transition state from stage I to II of disease. A total of 13 biological processes and molecular functions were related to the crucial genes.

**Conclusion**: In conclusion, the Upregulation of COL1A1, SERPINE1, PDGFRB, AURKA, TGFB1, and LGALS3 and downregulation of BRCA1 and TFRC in stage II of ESCC relative to stage I were pointed out as the key events which are associated with promotion of stage I to stage II transition.

**Keywords**: Esophageal cancer, expression profiles, Gene Expression Omnibus, Protein interaction.

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## Introduction

Esophageal cancer (EC) is a very poor prognosis malignancy with a high prevalence rate within the world, and the survival rate is very low even with treatment. EC is the sixth leading reason of cancer deaths all over the world. The highest-risk region, called the “esophageal cancer belt” includes portions of

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**Reprint or Correspondence**: **Alireza Ahmadzadeh**, *Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

**E-mail:** a.ahmadzadeh@sbmu.ac.ir
**ORCID ID:** 0000-0002-5474-6997

northern Iran, southern Russia, central Asian countries, and northern China (1-3). Esophageal adenocarcinoma and esophageal squamous cell carcinoma are the two main types of EC. Esophageal adenocarcinoma is more common than the other type. Based on pathological features, EC has 0-IV stages. As a rule, the lower the number of stages, the less the cancer has spread. A higher number means cancer has spread more (4, 5).

Understanding the molecular mechanism of the cancer process and discovering new biomarkers for early diagnosis is very important. Gene product and proteomic examinations via protein-protein interaction (PPI) network analysis are efficient tools commonly used in clinical research and finding diagnosis biomarker panels related to diseases. There are heterogeneous findings about gene expression changes related to the clinical outcome of esophageal cancer (6-8). Using PPI network analysis, genes or proteins concerned to a certain disease are retrieved and attributed in a collaborative structure with central elements (9). Hubs, bottlenecks, and hub-bottlenecks are the crucial elements of a PPI network, which are used frequently to detect molecular mechanisms of many diseases (10). Rezaei-tavirani et al. have introduced TP53, EGFR, AKT1, ERBB2, MYC, CCND1, CTNNB1, CDH1, and BCL2L1 as a candidate biomarker panel for esophageal adenocarcinoma (11). As Jiang S et al. reported, 21 biomarker candidates, including 7 individuals confirmed by literature, are introduced for esophageal squamous carcinoma. They suggested that RBPMS2, PDK4, IGK, SBSN, IFIT3, and HSPB6 are biomarkers of tumorigenesis for esophageal squamous carcinoma (12). Considering various types of reports, it seems more investigation is required to detect applicable biomarkers for esophageal cancer. The main goal of this study is to investigate the differences between stage I and II of esophageal squamous cell carcinoma (ESCC) via PPI network analysis to determined possible common and differential molecular features between both stages.

## Methods

### Data collection

To detect esophageal squamous cell carcinoma related genes, gene expression profiles (GEPs) of GSE161533 from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse161533) were considered to be analyzed. There are gene expression profiles of ESCC samples of patients in various stages of disease. Normal and para-tumor tissue of patients are presented as references. Data was evaluated via the GEO2R program to explore the significant DEGs.

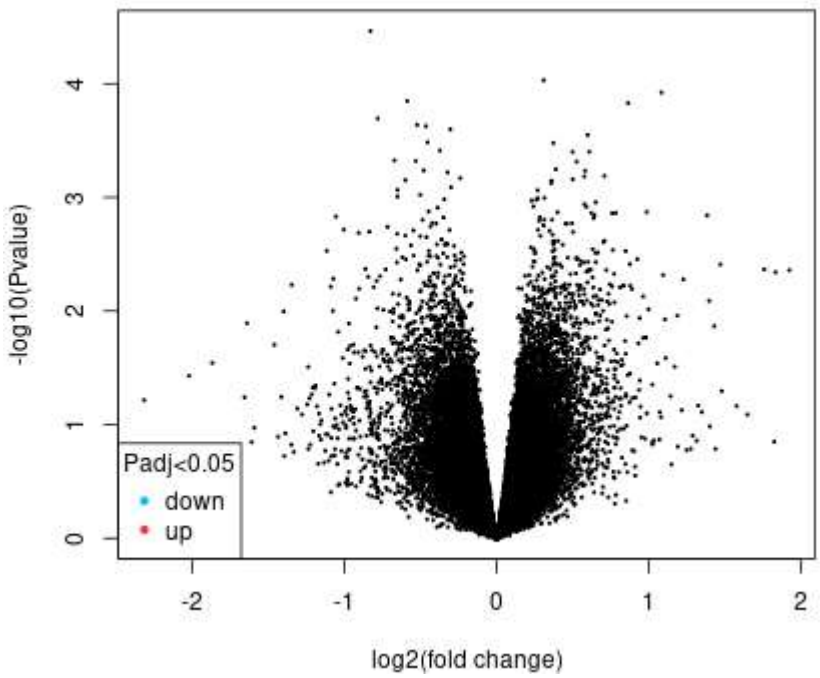


**Figure 1.** Volcano plot of GEPs comparison of tumor stage II versus tumor stage I comparison. There are no significant DEGs.

## Pre-evaluation analysis

Differences between GEPs of stage II and stage I were assessed via 2 procedures. First, the GEPs of stage II were compared with the GEPs of stage I as controls; second, the GEPs of stages II and I were compared separately with their normal controls. Results did not support the first way; therefore the second approach was applied to find the significant DEGs. The top 250 significant DEGs of each analysis were selected for analysis. The common DEGs between the two analyses were ignored and other genes were considered as differences between stages II and I of esophageal squamous cell carcinoma. The gene was excluded from more analysis if a DEG was common between the two analyses, but the difference between log fold change was < 0.6.

## PPI network analysis

The selected DEGs were included in the "protein query" of the STRING database via Cytoscape software v 3.7.2 to form a PPI network. The network was created via undirected edges and analyzed via the "Network analyzer" application of Cytoscape to detect the central genes. The network hubs were identified based on degree value > mean+2SD cutoff (13). To explore bottleneck nodes, the top 5% of genes based on betweenness centrality were determined as bottlenecks (14). The common hubs and bottlenecks were introduced as hub bottlenecks.

## Gene ontology analysis

The critical DEGs were assessed to find the related biological processes and molecular function via the ClueGO application of Cytoscape software. Network specificity more than medium was applied to detect the mapped biological terms.

## Statistical analysis

$P_{adj} < 0.05$ was considered to explore the significant DEGs. Degree cutoff > mean+2SD was applied to identified hub nodes. The top 5% of nodes based on betweenness centrality were determined as bottlenecks. The P-value of grouping: 0.05 and the correction test Bonferroni step-down were applied to analyze the significant enriched biological processes, molecular functions, and clusters.

## Results

Direct comparison between GEPs of tumor stages II and I cannot detect any significant DEGs (Figure 1). Results of the comparison of GEPs tumor stages II and I with their related controls are shown in Figures 2 and 3. As depicted in volcano plots, considerably significant DEGs separate the tumor samples from normal controls. After cleaning replicated data, 373 significant DEGs remained.

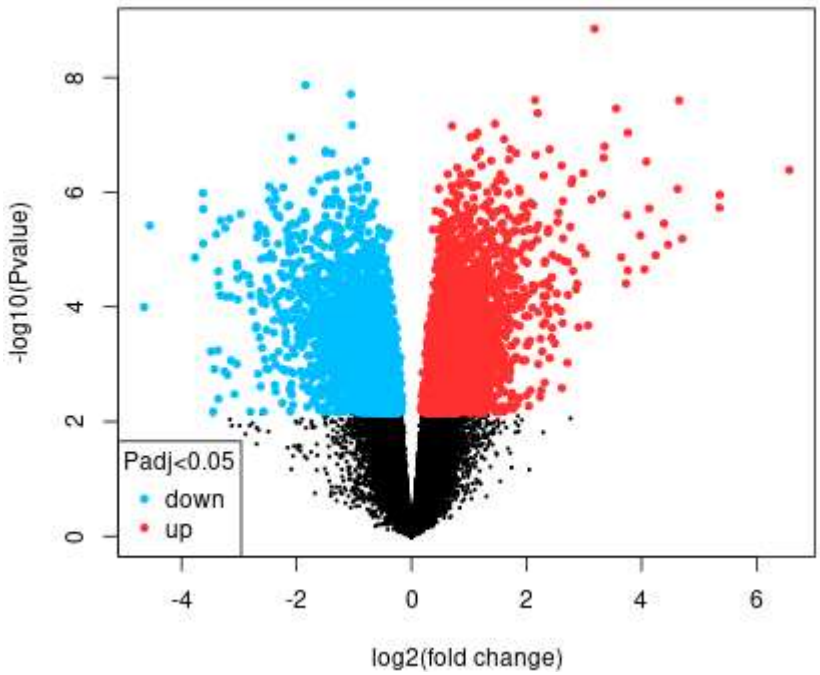


**Figure 2.** Volcano plot of GEPs comparison of tumor stage II versus normal II analysis. The significant DEGs are shown in blue and red.

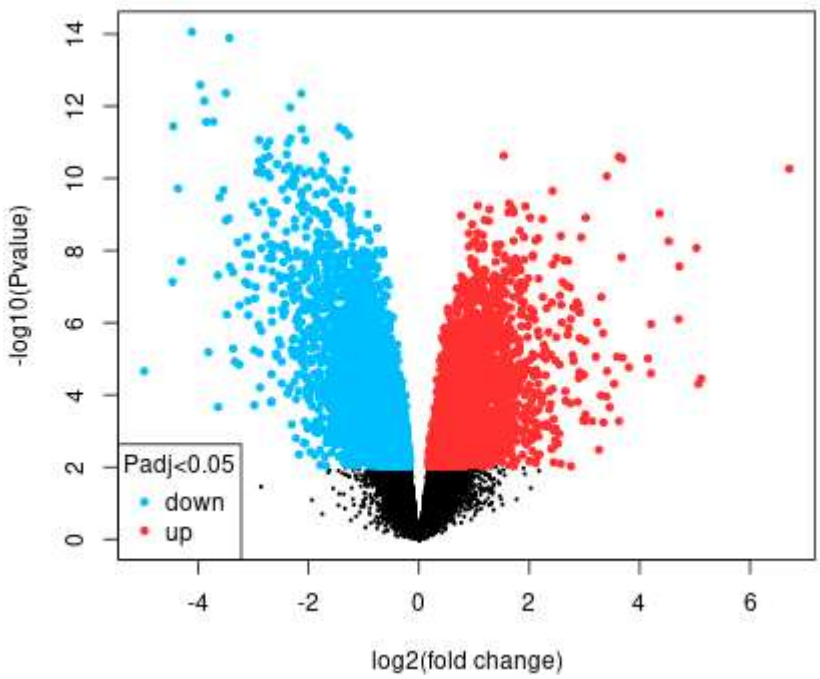


**Figure 3.** Volcano plot of GEPs comparison of tumor stage I versus normal I assessment. The significant DEGs are shown in blue and red.

**Table 1.** List of hub bottlenecks related to ESCC stage I; descriptions are extracted and summarized from the STRING database. K and BC stand for degree and betweenness centrality, respectively.

| No. | Gege | K | BC | STRING database description |
|---|---|---|---|---|
| 1 | LOX | 59 | 0.037 | Protein-lysine 6-oxidase; Regulator of Ras expression. May play a role in tumor suppression. Plays a role in the aortic wall architecture. |
| 2 | COL1A1 | 53 | 0.025 | Collagen alpha-1(I) chain; Type I collagen is a member of group I collagen. |
| 3 | SERPINE1 | 51 | 0.023 | Serpin peptidase inhibitor, clade E, member 1; It acts as 'bait' for tissue plasminogen activator, urokinase, protein C and matriptase-3/TMPRSS7. It may function as a major control point in the regulation of fibrinolysis. |
| 4 | TOP2A | 46 | 0.038 | Topoisomerase II makes double-strand breaks. Essential during mitosis and meiosis for proper segregation of daughter chromosomes. |
| 5 | PDGFRB | 46 | 0.019 | Platelet-derived growth factor receptor, beta polypeptide; It plays an essential role in the regulation of embryonic development, cell proliferation, survival, differentiation, chemotaxis and migration, and activation of several signaling cascades. |
| 6 | AR | 43 | 0.044 | Nuclear receptor subfamily 3 group C member 4; Steroid hormone receptors are ligand-activated transcription factors that regulate eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues. |
| 7 | AURKA | 43 | 0.032 | Serine/threonine-protein kinase aurora-A; It is associated with regulation of cell cycle progression, centrosome and the spindle microtubules during mitosis, regulation of KIF2A tubulin depolymerase activity, normal axon formation, and microtubule remodeling during neurite extension. |
| 8 | TGFBI | 43 | 0.020 | Transforming growth factor-beta-induced protein ig-h3; Plays a role in cell adhesion. May play a role in cell-collagen interactions. |
| 9 | LGALS3 | 42 | 0.031 | Lectin, galactoside-binding, soluble, 3; It is involved in endothelial cells migration, terminal differentiation of columnar epithelial cells during early embryogenesis, pre-mRNA splicing factor, acute inflammatory responses including neutrophil activation and adhesion, chemoattraction of monocytes macrophages, opsonization of apoptotic neutrophils, and activation of mast cells. |
| 10 | BRCA1 | 42 | 0.021 | Breast cancer type 1 susceptibility protein; It plays role in DNA repair, tumor suppressor function, centrosomal microtubule nucleation, normal cell cycle progression from G2 to mitosis. |
| 11 | CEBPB | 40 | 0.030 | CCAAT/enhancer binding protein (C/EBP), beta is involved in immune and inflammatory responses, adipogenesis, gluconeogenic pathway, liver regeneration, and hematopoiesis, female reproduction, and osteoclastogenesis. |
| 12 | CTSB | 37 | 0.036 | APP secretase; Thiol protease which is believed to participate in intracellular degradation and turnover of proteins. Has also been implicated in tumor invasion and metastasis. |
| 13 | SERPINH1 | 36 | 0.016 | Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1); Binds specifically to collagen. |
| 14 | TFRC | 35 | 0.040 | Transferrin receptor protein 1; Transferrin receptor is necessary for development of erythrocytes and the nervous system. Positively regulates T and B cell proliferation through iron uptake. |

The PPI network was constructed, including 365 recognized significant DEGs and 2284 edges. The degree cutoff to determine hubs was known as 33. LOX, COL1A1, SERPINE1, COL1A2, PDGFRB, TOP2A, AR, AURKA, COL5A1, MMP3, TGFBI, BRCA1, LGALS3, THY1, COL4A1, CEBPB, BGN, COL4A2, CTSB, SERPINH1, CTHRC1, TFRC, and MMP12 were identified as hub genes. In the other hand, AR, TFRC, TOP2A, LOX, CTSB, AURKA, LGALS3, CEBPB, MAPT, COL1A1, SERPINE1, BRCA1, TGFBI, PDGFRB, CLPB, NCF2, SERPINH1, SORT1, and KIF18B were recognized as bottleneck nodes. A list of 14 hub-bottleneck genes is presented in Table 1. As shown in Table 1, descriptions of the 14 hub-bottleneck genes were extracted from the STRING database (https://string-db.org/cgi/input?sessionId=bZhPmsmWoGKB&input_pag

**Table 2.** Presenting logFC for hub-bottleneck genes in tumor stages I and II analyses. The bold individuals are extracted from the genes not included in the 250 top significant DEGs.

| No. | Gege | LogFC | |
|---|---|---|---|
| | | Tumor stage II analysis | Tumor stage I analysis |
| 1 | LOX | 2.195 | 1.903 |
| 2 | COL1A1 | 4.132 | 3.537 |
| 3 | SERPINE1 | 2.538 | 1.635 |
| 4 | TOP2A | 1.4 | 1.747 |
| 5 | PDGFRB | 1.1 | 0.642 (not significant) |
| 6 | AR | -1.17 | -1.430 |
| 7 | AURKA | 1,284 | 0.319 (not significant) |
| 8 | TGFBI | 2.162 | 1.542 |
| 9 | LGALS3 | -0.02 (not significant) | -0.754 |
| 10 | BRCA1 | 0.02 (not significant) | 1.065 |
| 11 | CEBPB | 1.438 | 1.595 |
| 12 | CTSB | 0.794 | 0.603 |
| 13 | SERPINH1 | 2.202 | 1.822 |
| 14 | TFRC | 0.416 | 1.841 |

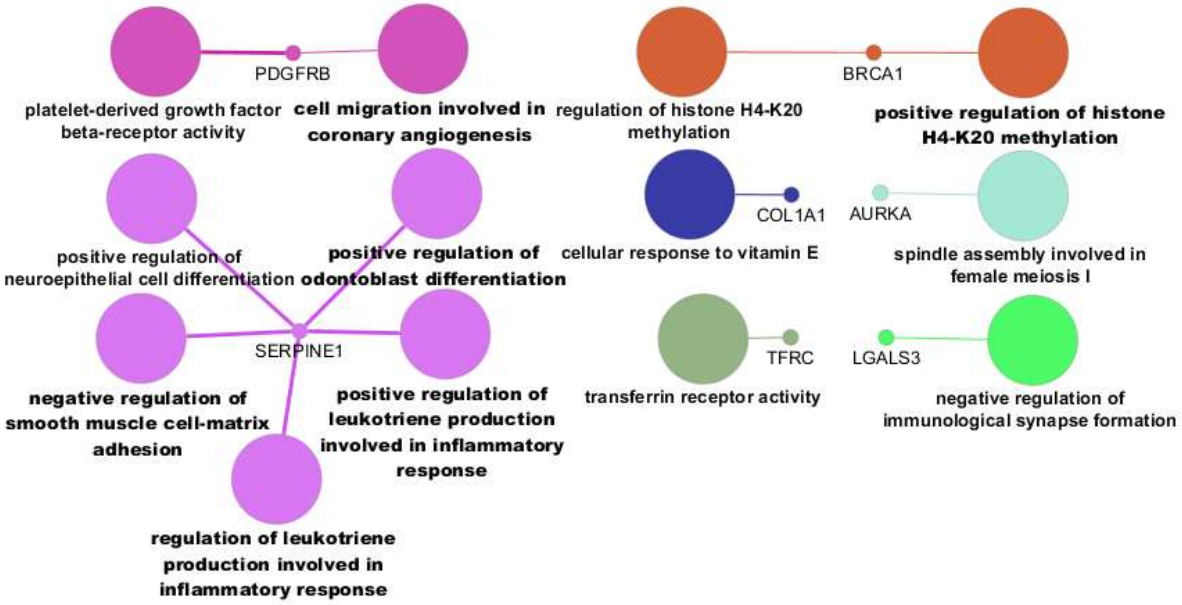


**Figure 4.** Results of gene ontology enrichment of the critical DEGs. COL1A1 is not included in the network. Small circles stand for associated genes. The related biological processes and molecular functions are presented via larger circles.

e_show_search=on). Since the top 250 significant DEGs of each analysis were evaluated, amounts of gene expression change of hub-bottleneck DEGs that were not included in 250 significant DEGs were searched in the other reported genes for analyses. Results are shown in Table 2. Based on the results of Table 2, LOX, TOP2A, AR, CEBPB, CTSB, and SERPINH1, which had no significant difference in gene expression in tumor stages II and I, were ignored from more investigation, and the rest were considered as critical genes. The critical DEGs, including COL1A1, SERPINE1, PDGFRB, AURKA, TGFBI, LGALS3, BRCA1, and TFRC, were enriched to find the biological processes and molecular function (Figure 4).

## Discussion

Volcano plot visualization of the expressed genes (Figures 1-3) revealed significant differences between GEPs of patients with stage 1 the stage II individuals. Jin Zhou et al. investigation indicates that gene expression change can be considered a tool to assess stage transition in esophagus cancer (15). As depicted in Table 1, 14 central DEGs are associated with transitioning from stage I to stage II. More analysis showed that COL1A1, SERPINE1, PDGFRB, AURKA, TGFBI, LGALS3, BRCA1, and TFRC are the critical central genes that discriminate patients with stage II from individuals with stage I. The connection between the crucial genes and related biological terms is depicted in Figure 4.

Li J et al. study revealed that COL1A1 has been up-regulated in ESCC tissues compared to normal controls (16). As shown in Table 2, COL1A1 is extremely upregulated in stages I and II in ESCC patients. However, the rate of its upregulation in stage II is more than in stage I. It seems the rate of its upregulation is positively correlated to cancer progression. Lv J et al. have suggested COL1A1 as a potential biomarker for distinguishing esophageal adenocarcinoma (17). SERPINE1 is another critical gene. Based on the report of Klimczak Bitner AA et al., SERPINE1, MMP9, and miR-134 are prognostic factors in esophageal cancer (18). Results of Table 2 indicate SERPINE1 is upregulated, and its overexpression is associated with cancer development.

The third critical gene is PDGFRB, which is upregulated in stage II, while its upregulation in stage I is not significant. Upregulation of PDGFRB in esophageal cancer is confirmed via literature (19). PDGFRB also is highlighted as a prognostic biomarker in gastric cancer (20). Like COL1A1 and SERPINE1, upregulation of PDGFRB is correlated to the progression of esophageal cancer. The other crucial DEG is LGALS3, which is downregulated in stage I. Its expression change in stage II is not considerable. As described in Table 1, lectin galactoside-binding soluble 3 plays a role in endothelial cell migration, pre-mRNA splicing factor, terminal differentiation of columnar epithelial cells during early embryogenesis, chemoattraction of monocytes macrophages, acute inflammatory responses including neutrophil activation and adhesion, activation of mast cells, and opsonization of apoptotic neutrophils. LGALS3 gene encodes galectin-3 protein associated with the development and malignancy of esophageal squamous cell carcinoma (21, 22). It seems the production of galectin-3 increases along with the progress of ESCC.

Like the mentioned critical genes, the amount of AURKA expression is increased, accompanied by cancer development. There is evidence about the key role of AURKA in the growth of ESCC. Overexpression of AURKA in upper gastrointestinal adenocarcinomas is confirmed via investigations (23). Various functions related to the essential cellular activity are counted for AURKA in Table 1. TGFB1 is the other crucial gene upregulating in stage II relative to stage I. Ozawa D et al. published a document about elevated expression of TGFB1 in ESCC tissues. They suggested TGFB1 as a powerful biomarker of poor prognosis and hematogenous reappearance of ESCC (24).

BRCA1 is downregulated in stage II relative to stage I. Its role in DNA repair, centrosomal microtubule nucleation, tumor suppressor function, and normal cell cycle progression from G2 to mitosis is highlighted in Table 1. Studies have shown inactivation of BRCA1 as a tumor suppressor gene might be a primary occurrence during esophageal carcinogenesis (25). Wei B et al. have recommended BRCA1 mRNA expression as a prognostic marker in molecular staging for personalized treatment in patients with progressive ESCC (26). The last critical gene is TFRC, downregulated in stage II versus stage I of ESCC patients. Wada S et al. pointed out TFRC as a prognostic factor for ESCC (27). As discussed, experimental validation is required to select the biomarker panel members among the introduced critical genes to detect the possible drug targets or diagnostic markers.

## Conclusion

In conclusion, eight genes, including COL1A1, SERPINE1, PDGFRB, AURKA, TGFBI, LGALS3, BRCA1, and TFRC, are suggested as the crucial individuals associated with stage I to stage II of ESCC. Upregulation of COL1A1, SERPINE1, PDGFRB, AURKA, TGFB1, and LGALS3 and downregulation of BRCA1 and TFRC in stage II of ESCC versus stage I were explored. More investigations can confirm and limit the finding to discover a suitable prognostic biomarker. Assessment of drug affinity and binding of the introduced critical genes can provide beneficial information about possible drug targets.

## Conflict of interests

The authors declare that they have no competing interests.

## Acknowledgment

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