Am J Transl Res 2023;15(3):1550-1568
www.ajtr.org /ISSN:1943-8141/AJTR0145018

Original Article

Estimation of hub genes and exploration of multi-omics level alterations in the landscape of lung adenocarcinoma

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Received June 28, 2022; Accepted January 5, 2023; Epub March 15, 2023; Published March 30, 2023

Abstract: Objectives: Lung adenocarcinoma (LUAD) is recognized as one of the most prevalent and deadliest malignancies around the globe. The molecular mechanisms behind LUAD have not been fully elucidated. This study was launched to explore LUAD-associated hub genes and their enriched pathways using bioinformatics methods. Methods: Information on GSE10072 was retrieved from the Gene Expression Omnibus (GEO) database and analyzed via the Limma package-based GEO2R tool to obtain the top 100 differentially expressed genes (DEGs) in LUAD. The protein-protein interaction (PPI) network of the DEGs was drawn using the STRING website and was shifted into Cytoscape to screen the top 6 hub genes via the CytoHubba application. Furthermore, the expression analysis and validation of hub genes in LUAD samples and cell lines were done using UALCAN, OncoDB, and GENT2 databases. Moreover, OncoDB was also used for analyzing hub gene DNA methylation levels. In addition, cBioPortal, GSEA tool, Kaplan-Meier (KM) plotter, Enrichr, CancerSEA, and DGIdb were performed to explore some other important aspects of hub genes in LUAD. Results: We identified Interleukin 6 (IL6), Collagen, type I, alpha 1 (COL1A1), TIMP metallopeptidase inhibitor 1 (TIMP1), CD34 molecule (CD34), Decorin (DCN), and Secreted Phosphoprotein 1 (SPP1) genes as the hub genes in LUAD, out of which IL6, CD34, and DCN were significantly down-regulated while COL1A1, TIMP1, and SPP1 were significantly up-regulated in LUAD cell lines and samples of diverse clinical variables. In this study, we also documented some important correlations between hub genes and other parameters such as DNA methylation, genetic alterations, Overall Survival (OS), and 14 important states at the single cell level. Lastly, we also identified hub genes associated with the ceRNA network and 11 important chemotherapeutic drugs. Conclusion: We identified 6 hub genes involved in the development and progression of LUAD. These hub genes can also be helpful in the accurate detection of LUAD and provide novel ideas for treatment.

Keywords: Lung adenocarcinoma, hub genes, biomarkers, chemotherapeutic drugs

Introduction

Lung cancer, due to its alarmingly increasing prevalence, is one of the most life threatening malignant tumors around the globe [1]. Based on the cancer stats provided by the World Health Organization (WHO), the prevalence and mortality rate of lung cancer were ranked first worldwide [2]. In the year 2021, approximately 236,740 adults (including both male and females) were diagnosed with lung cancer alone in the United States [3]. Moreover, in the year of 2021, around 2,206,771 individuals (including both males and female) were diagnosed with lung cancer worldwide. The described lung cancer statistics include both lung adenocarcinoma (LUAD) and Non-Small Cell Lung Cancer (NSCLC). NSCLC mainly consists of three histological types, namely adenocarcinoma, large cell carcinoma, and squamous cell carcinoma [4]. In most countries, adenocarcinoma was found to be the most frequently reported lung

cancer compared to other subtypes. LUAD development is a multi-step, complicated process involving dysregulation and changes in the genetic makeup of various important genes [4].

B-cell lymphoma 2 (BCL-2) participates in the development and progression of LUAD by preventing apoptosis [5]. Moreover, it is also known that in exon 21 of the EGFR gene, a L858R mutation (point) and complete deletion of this exon are associated with the induction of cell proliferation, inhibition of apoptosis, angiogenesis, and metastasis in LUAD [6]. Elevated expressions of other different important genes such as MACC1, SR-B1, and KIF2A have also been found to be closely related to the development and poor prognosis of LUAD [7, 8]. *Wang et al.,* identified and validated the expression of 7 hub genes, including BIRC5, CDC20, MCM4, FOXM1, RFC4, CDC25C, and GTSE1, which play important roles in the development and metastasis of LUAD [9]. In the Chinese population, *Xie et al.,* explored 8 hub genes (ADCY4, AVPR2, CALCRL, RAMP3, RXFP1, RAMP2, VIPR1, and ADRB1) associated with the occurrence and metastasis of LUAD [10]. Moreover, *Wu et al.,* also explored and validated 4 hub genes (HLF, CHRDL1, SELENBP1, and TMEM163) via bioinformatics analysis and wet-lab experiments as diagnostic and prognostic biomarkers of LUAD [11]. However, to our knowledge, the underlying mechanisms governing LUAD development are still not well proven, making it difficult to diagnosis and treat LUAD. Therefore, the elucidation of molecular mechanisms governing LUAD development and progression is very urgent and critical.

During the last decade, microarray technology has improved significantly [12]. Gene expression microarrays have been proven to be the most reliable tool for analyzing differentially expressed genes (DEGs) associated with the development and progression of LUAD [13, 14]. Many previous studies on LUAD have not integrated gene expression data across-platforms [15] to explore the molecular basis of LUAD. Therefore, the utility of cross-platform analysis will increase the reliability of the analysis results.

In the current research, LUAD-associated DEGs were identified from the GSE10072 dataset [16] using the GEO2R tool. For this purpose, this dataset was taken from the Gene Expression Omnibus (GEO) [17]. Later on, the top 6 hub genes were further explored via the degree method. Finally, we employed a variety of bioinformatics methods on the hub genes to uncover evidence and clues related to the molecular mechanisms governing LUAD development and progression.

Methodology

Dataset retrieval

The GEO database (https://www.ncbi.nlm.nih.gov/geo/) [18], which is very famous for providing experimental gene expression data, was mined in this study to retrieve the GSE10072 dataset, comprising of 58 LUAD and 49 normal lung samples. The platform of this dataset was the Affymetrix Human Genome U95A Version 2 Array.

Analysis of the GSE10072 dataset and the DEGs identification

The Limma package-based GEO2R tool was used in this study to analyze GSE10072 dataset to identify the top 100 DEGs in LUAD samples relative to controls [18]. In the GEO2R tool, a t-test and the Benjamin-Hochberg method were used to measure the *P*-value and FDR of the DEGs. The whole process of DEGs screening was done in accordance with the principal standards, i.e., $P<0.05$ and $logFC>1$.

Protein interaction analysis and the hub genes' recognition

In this study, the protein-protein interaction network of identified DEGs was exploited via the STRING database (https://string-db.org/) [19]. During the analysis, the cutoff score for the protein interaction network was fixed as >0.9 to accomplish a network of the DEGs. Then, the obtained protein interaction network from the STRING database was visualized using Cytoscape software v3.8.1. Later on, based on the degree method, the top six hub genes in the constructed network were recognized via the CytoHubba application of the Cytoscape software.

Hub genes' expression level in LUAD via UALCAN

UALCAN (http://ualcan.path.uab.edu/) is acknowledged as a reliable tool for gene expression profiling analysis across thousands of

tumor and normal samples. This tool attained RNA expression data from TCGA and GTEx ventures and we presented this data in the form of box plots [20]. We analyzed hub gene expression (mRNA and protein) across the LUAD cohort via this tool.

Hub genes' expression validation via GENT2 and OncoDB

Then, to further validate hub genes' expression across LUAD tissues and cell lines, we employed the OncoDb (http://oncodb.org/) and GENT2 (http://gent2.appex.kr) databases. These databases also provide expression analysis results in the form of box plots [21, 22].

DNA methylation analysis

OncoDB is a user-defined friendly database, which is available online to carry out gene expression and methylation analysis across thousands of cancer patients suffering from 33 major cancer subtypes [23]. In our research, this resource was used to analyze the DNA methylation of hub genes across LUAD patients.

cBioPortal data analysis

cBioPortal (https://www.cbioportal.org/) provides easy access to cancer genomic data for multidimensional *in silico* analysis [24]. We used this database to evaluate the genomic mutations of the hub genes across LUAD.

Functional enrichment analysis

In this research, functional enrichment, including GO and KEGG analysis of the hub genes was performed using the GSEA tool with a *P*-value of P<0.05. This tool identified KEGG and GO terms based on the biological phenomena of the studied protein or gene list [25].

OS analysis of hub genes

The Kaplan-Meier (KM) plotter (https://kmplot.com/) has been acknowledged to explore the effect of human coding genes on the OS of 21 cancer subtypes [26]. This tool calculates the hazard ratio (HR) and 95% confidence interval via the R and Bioconductor package. In our study, this analysis was performed to evaluate the effect of hub genes on the OS of LUAD patients.

ceRNA network of the hub genes'

In our study, the ceRNA network of the identified hub genes was predicted using the Enrichr database (http://amp.pharm.mssm.edu/Enrichr) [27].

CancerSEA analysis

CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA/) was developed for decoding Pearson correlations between gene(s) of interest and 14 different functional states at the single-cell level in human cancers [28]. Herein, we utilized CancerSEA to explore the correlations between hub genes and the above-mentioned functional states in LUAD.

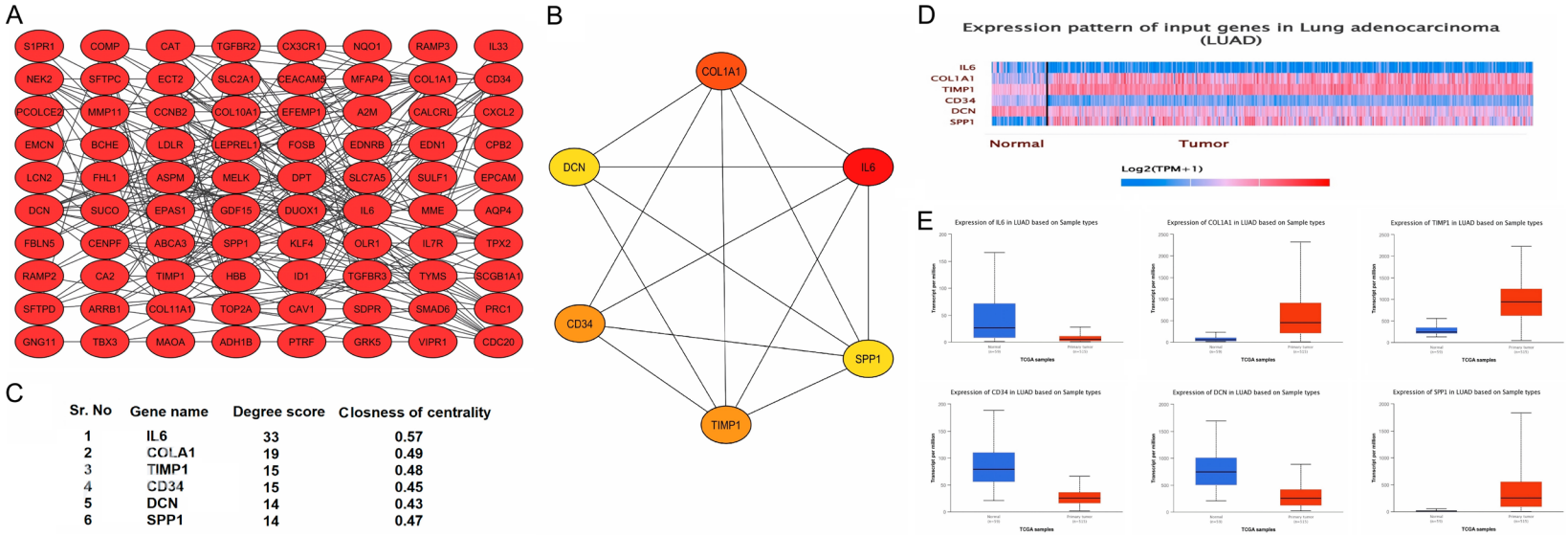
Hub genes' associated drugs

The identified hub genes can be promising therapeutic targets, and in this view, we conducted the DGIdb analysis to identify hub genes' associated drugs. This database provides details on drugs targeting hub genes from different reliable sources [29].

Results

Identification of the DEGs, PPI network construction, screening of the hub genes and their expression analysis

By analyzing the GSE10072 dataset, the top 100 DEGs were initially retrieved successfully. Later on, a PPI network of the DEGs containing 100 nodes (genes) and 1134 edges (interactions) was constructed via STRING (**Figure 1A**). In the constructed PPI, the *P* value of PPI enrichment was <1.0 × 10-16. Moreover, 6 DEGs, showing the highest degree scores of centrality were considered as the hub genes by analyzing the PPI network via the plugin CytoHubba application of Cytoscape (**Figure 1B**, **1C**). Results of the analysis showed that the top 6 hub genes with the highest degree of centrality were IL6 (Interleukin 6), COL1A1 (Collagen, type I, alpha 1), TIMP1 (TIMP Metallopeptidase Inhibitor 1), CD34 (CD34 Molecule), DCN (Decorin), and SPP1 (Secreted Phosphoprotein 1). Next, the mRNA expression analysis of IL6, COL1A1, TIMP1, CD34, DCN, and SPP1 across LUAD and non-tumor lung tissues was carried out via UALCAN. As highlighted in **Figure 1D**, **1E**, the mRNA expression of IL6 (**Figure 1D**, **1E**), CD34



C

Sr. No	Gene name	Degree score	Closness of centrality
1	IL6	33	0.57
2	COL1A1	19	0.49
3	TIMP1	15	0.48
4	CD34	15	0.45
5	DCN	14	0.43
6	SPP1	14	0.47

Figure 1. Interaction network of the top 100 DEGs extracted from GSE10072, top 6 hub genes identification via the degree method, and the expression profiling using UALCAN. (A) Interaction network of top 100 DEGs, (B, C) Top 6 identified hub genes based on degree method, and (D, E) mRNA expression profiling of hub genes across LUAD samples and normal controls. A *p* value score of less than <0.05 was considered significant.

(**Figure 1D**, **1E**), and DCN (**Figure 1D**, **1E**) were significantly down-regulated while the mRNA expression of COL1A1 (**Figure 1D**, **1E**), TIMP1 (**Figure 1D**, **1E**), and SPP1 (**Figure 1D**, **1E**) were significantly up-regulated in LUAD samples as compared to non-tumor lung tissues (**Figure 1D**, **1E**).

Hub genes' expression across various subgroups of LUAD

Via UALCAN analysis, the lower expression of 3 hub genes, including IL6, CD34, and DCN, while the higher expression of other 3 hub genes including CD34, DCN, and SPP1, were also found to be linked with different LUAD subgroups based on different pathological factors including cancer stage, race, gender, age, race, smoking habit, and nodal metastasis (**Figure 2**). The *P* values among all analyzed subgroups and normal sample groups were significant (<0.05) (**Figure 2**).

Hub genes' protein level expression analysis

Furthermore, the hub genes' protein expression levels across LUAD samples relative to controls were analyzed through UALCAN. Obviously, the levels of IL6, CD34, and DCN protein expression were significantly lowered in LUAD samples, while the levels of CD34, DCN, and SPP1 proteins were significantly higher in LUAD samples relative to normal controls (**Figure 3**). The *P* values among all analyzed LUAD and normal sample groups were less than 0.05 (**Figure 3**). In a nutshell, our present results highlighted that mRNA and protein expression of IL6, CD34, and DCN were down-regulated while mRNA and protein expression of the CD34, DCN, and SPP1 were up-regulated in LUAD samples than the controls.

Verification of hub genes' expression across LUAD cell lines and different independent LUAD cohorts

In our study, we confirmed the mRNA expression of hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1) in LUAD cell lines via GENT2 analysis and different independent cohorts via the OncoDB database, with in-house 540 LUAD and 59 normal lung samples. The results of the expression verification analysis were consistent with the results of UALCAN, i.e., these databases also highlighted that mRNA expression of IL6, CD34, and DCN were down-regulated while the mRNA expression of CD34, DCN, and SPP1 were up-regulated in LUAD cell lines and patients from different cohorts (**Figure 4**).

DNA methylation analysis of the hub genes

To evaluate the link between hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1) DNA methylation and expression levels across TCGA LUAD datasets, we quantified the methylation levels of the hub genes by averaging CpG beta values in the gene body and promoter regions via OncoDB. Later on, the Wilcoxon test was applied to measure the levels of significance among differentially methylated statuses between LUAD and control samples. Via analysis results, we found significant hypermethylation of all hub genes, including IL6, COL1A1, TIMP1, CD34, DCN, and SPP1 in LUAD samples relative to controls (**Figure 5**).

Genomic alteration analysis

It is acknowledged that genomic alterations have a close association with tumorigenesis [30]. To explore the genomic alterations of hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1) across LUAD samples, we applied the cBioPortal analysis using the pan-cancer atlas dataset. Results showed that the genomic alteration frequency of CD34 was 6%, ranked 1st, followed by COL1A1 (4.1%), IL6 (3.6%), TIMP1 (1.6%), DCN (1.3%), and SPP1 (1%). Our results also showed that all hub genes (except TIMP1) exhibited "DNA mutation" genomic alteration as the most abundant genomic alteration in hub genes (**Figure 6A**). As highlighted in **Figure 6B**, the most frequently observed mutations in hub genes are K94N (IL6), P871S (COL1A1), R315L (CD34), S214C (DCN), and D109A (SPP1).

Functional annotation of the hub genes

The functional annotation of hub genes was done via the GSEA analysis. For this purpose, the hub genes were categorized into biological process (BP) terms, molecular function (MF) terms, cellular component (CC) terms (**Figure 7A**), and pathway terms (**Figure 7B**). Results of the analysis showed that BP terms of the hub genes mainly involved were: cell communication, metabolic process, and cellular component organization, etc. (**Figure 7A**); the MF

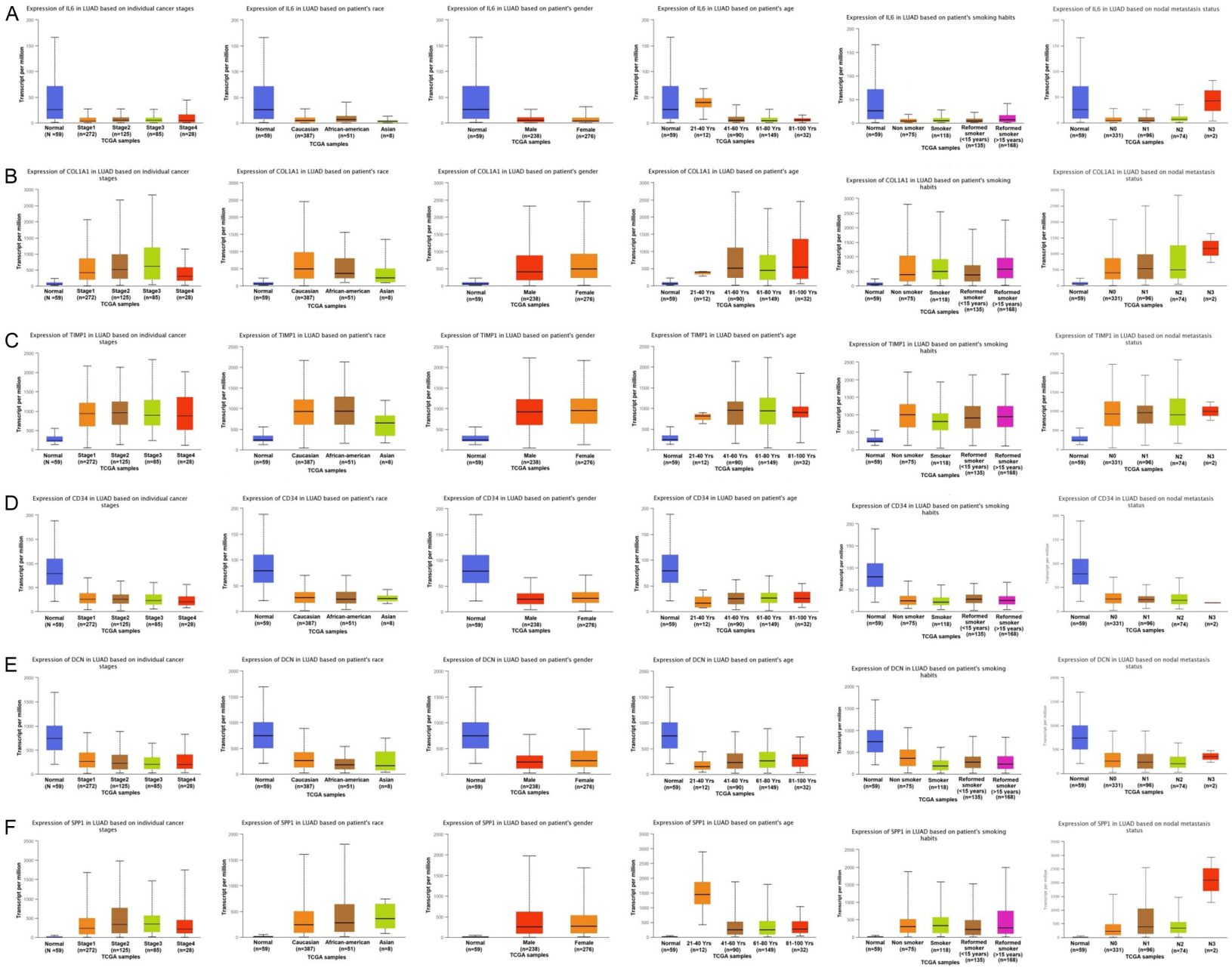

A
Expression of IL6 in LUAD based on individual cancer stages
Expression of IL6 in LUAD based on patient's race
Expression of IL6 in LUAD based on patient's gender
Expression of IL6 in LUAD based on patient's age
Expression of IL6 in LUAD based on patient's smoking habits
Expression of IL6 in LUAD based on nodal metastasis status
B
Expression of COL1A1 in LUAD based on individual cancer stages
Expression of COL1A1 in LUAD based on patient's race
Expression of COL1A1 in LUAD based on patient's gender
Expression of COL1A1 in LUAD based on patient's age
Expression of COL1A1 in LUAD based on patient's smoking habits
Expression of COL1A1 in LUAD based on nodal metastasis status
C
Expression of TIMP1 in LUAD based on individual cancer stages
Expression of TIMP1 in LUAD based on patient's race
Expression of TIMP1 in LUAD based on patient's gender
Expression of TIMP1 in LUAD based on patient's age
Expression of TIMP1 in LUAD based on patient's smoking habits
Expression of TIMP1 in LUAD based on nodal metastasis status
D
Expression of CD34 in LUAD based on individual cancer stages
Expression of CD34 in LUAD based on patient's race
Expression of CD34 in LUAD based on patient's gender
Expression of CD34 in LUAD based on patient's age
Expression of CD34 in LUAD based on patient's smoking habits
Expression of CD34 in LUAD based on nodal metastasis status
E
Expression of DCN in LUAD based on individual cancer stages
Expression of DCN in LUAD based on patient's race
Expression of DCN in LUAD based on patient's gender
Expression of DCN in LUAD based on patient's age
Expression of DCN in LUAD based on patient's smoking habits
Expression of DCN in LUAD based on nodal metastasis status
F
Expression of SPP1 in LUAD based on individual cancer stages
Expression of SPP1 in LUAD based on patient's race
Expression of SPP1 in LUAD based on patient's gender
Expression of SPP1 in LUAD based on patient's age
Expression of SPP1 in LUAD based on patient's smoking habits
Expression of SPP1 in LUAD based on nodal metastasis status
Transcript per million
TCGA samples

Figure 2. Hub genes' mRNA expression across various sub groups of the LUAD patients. (A) IL6 hub gene expression across cancer stage, race, gender, age, smoking condition, and nodal metastasis subgroups of LUAD, (B) COL1A1 hub gene expression across cancer stage, race, gender, age, smoking condition, and nodal metastasis subgroups of LUAD, (C) TIMP1 hub gene expression across cancer stage, race, gender, age, smoking condition, and nodal metastasis subgroups of LUAD, (D) CD34 hub gene expression across cancer stage, race, gender, age, smoking condition, and nodal metastasis subgroups of LUAD, (E) DCN hub gene expression across cancer stage, race, gender, age, smoking condition, and nodal metastasis subgroups of LUAD, and (F) SPP1 hub gene expression across cancer stage, race, gender, age, smoking condition, and nodal metastasis subgroups of LUAD. A *p* value score of less than <0.05 was considered significant. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1.

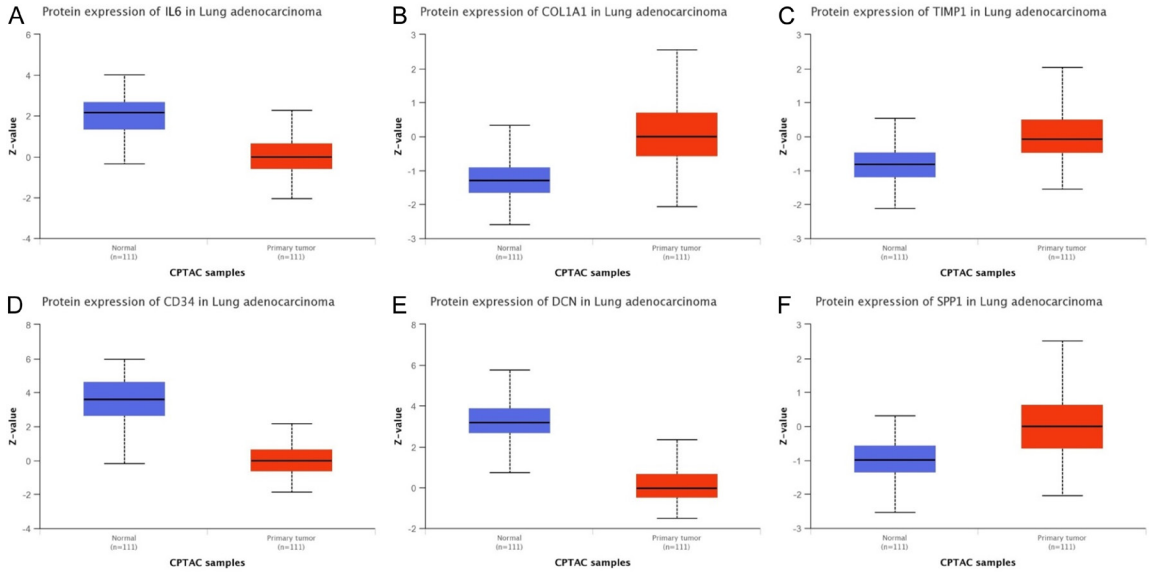


Figure 3. Hub genes' protein expression across LUAD and normal samples. (A) IL6 protein expression, (B) COL1A1 IL6 protein expression, (C) TIMP1 IL6 protein expression, (D) CD34 IL6 protein expression, (E) DCN IL6 protein expression, and (F) SPP1 IL6 protein expression. A *p* value score of less than <0.05 was considered significant. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1.

terms of the hub genes mainly involved: protein containing complex, extracellular spaces, and endomembrane system, etc. (**Figure 7A**); the CC terms of the hub genes mainly involved: protein binding, ion binding, structural molecule activity, and carbohydrate binding, etc. (**Figure 7A**); the pathway terms of the hub genes mainly involved: immune system, and degradation of extracellular matrix (**Figure 7B**).

Survival analysis

Next, to evaluate the relationships among hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1) and LUAD, the OS analysis of these genes was carried out via the Kaplan-Meier plotter. As highlighted in **Figure 8**, the higher expressions of COL1A1, TIMP1, and SPP1, and lower expressions of IL6, CD34, and DCN across LUAD patients were significantly associated with poor OS (**Figure 8**).

The ceRNA network of the hub genes was constructed

During this step, we evaluated the top 10 experimentally validated miRNAs in relationship with each hub gene from Enrichr (**Figure 9**). This step also includes the exploration of 10 experimentally validated lncRNAs from Enrichr (**Figure 9**). Finally, we speculate that the lncRNA-miRNA-mRNA network of the lncRNAs and miRNAs in relation to hub genes can be used to evaluate the development of LUAD at the molecular level in more depth.

Single-cell functional analysis

CancerSEA was used here to further explore the roles of hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1) in LUAD at the single cell level. As shown in **Figure 10**, the identified hub genes were found to be associated (posi-

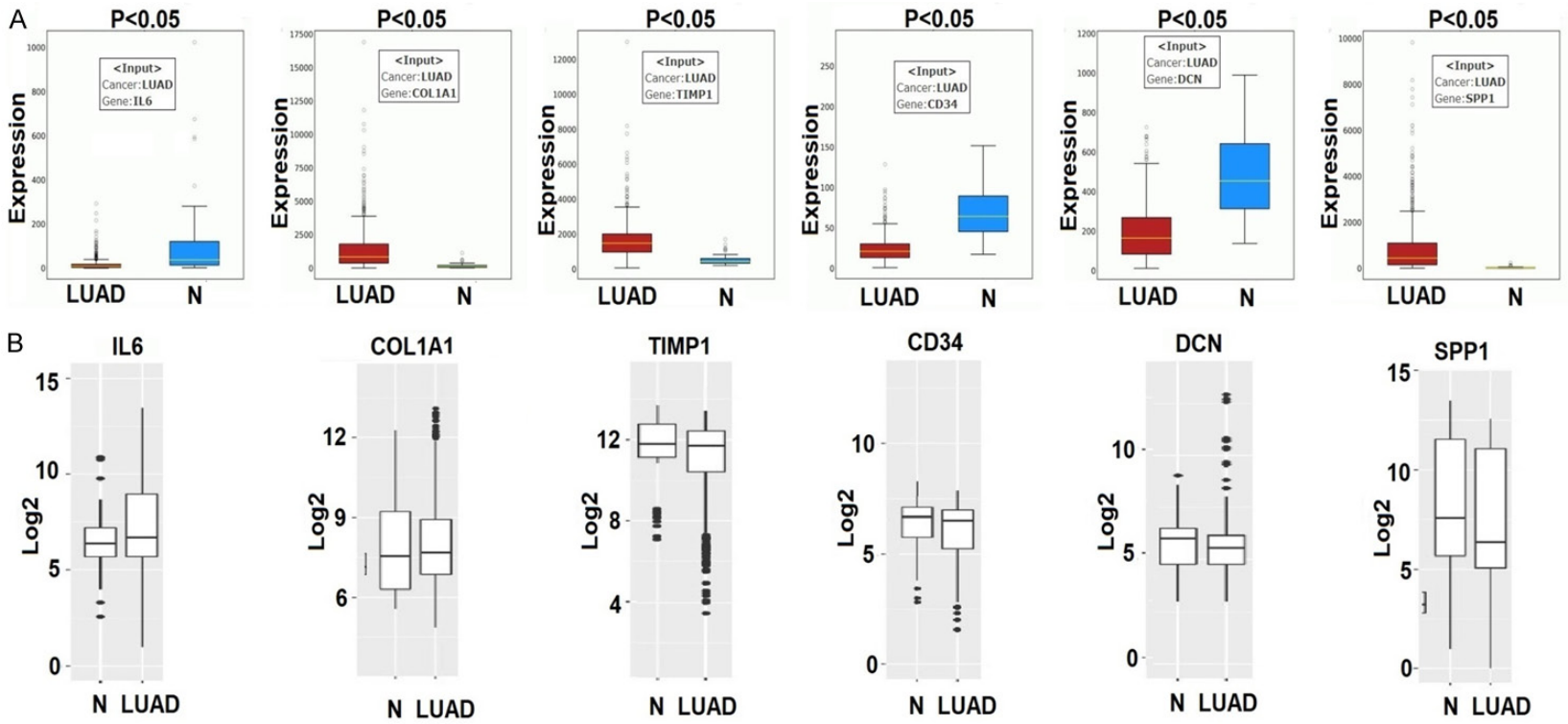


Figure 4. Expression evaluation of 6 hub genes across new TCGA cohorts of the LUAD patients, and cell lines via OncoDB and GENT2 databases. (A) Expression evaluation of 6 hub across new TCGA cohort of LUAD patients via oncoDB database, and (B) Expression evaluation of 6 hub across LUAD cell line via GENT2 database. A *p* value score of less than <0.05 was considered significant. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1, LUAD = Lung adenocarcinoma.

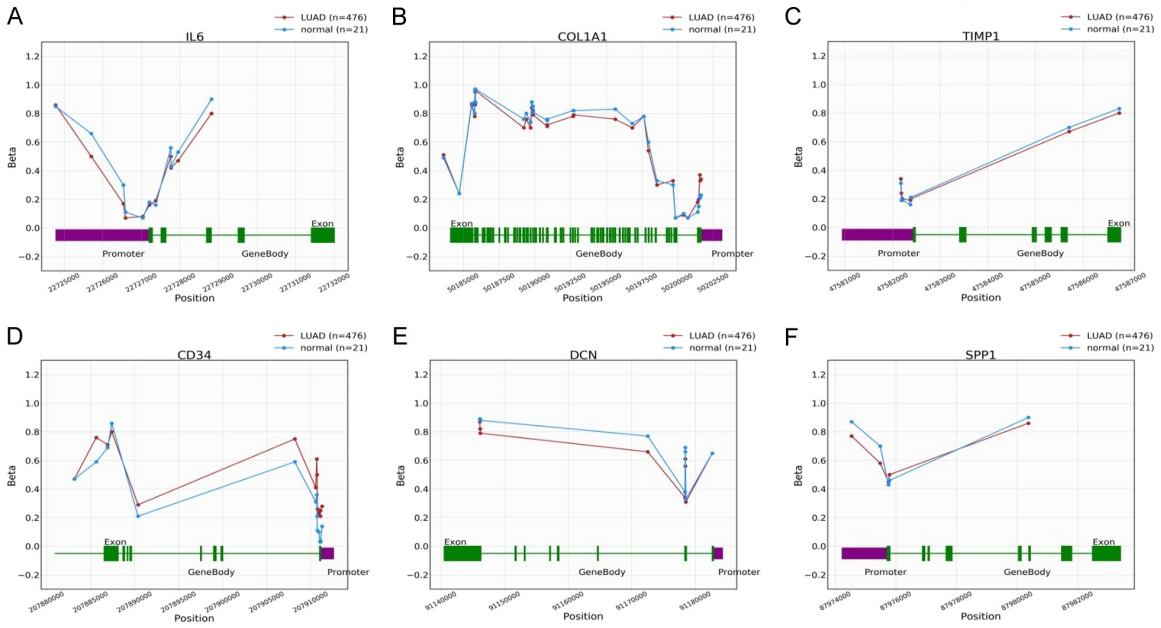


Figure 5. DNA methylation level evaluation of 6 hub genes across LUAD samples paired with controls via OncoDB databases. (A) Results of IL6 DNA methylation analysis, (B) Results of COL1A1 DNA methylation analysis, (C) Results of TIMP1 DNA methylation analysis, (D) Results of CD34 DNA methylation analysis, (E) Results of DCN DNA methylation analysis, and (F) Results of SPP1 DNA methylation analysis. A *p* value score of less than <0.05 was considered significant. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1, LUAD = Lung adenocarcinoma.

tively or negatively) with 14 different states (**Figure 10A**). However, hub gene expression was significantly positively correlated with only Quiescence, Invasion, Metastasis, and Hypoxia states (**Figure 10B**).

Hub genes associated drugs

Via the DGIdb database, we next explored the drug-gene interactions of the hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1). In total, 11 drugs were identified for potentially treating LUAD (**Table 1**). The reliable target hub genes of these drugs were IL6, COL1A1, and SPP1. Our model of hub-gene drug interaction showed that inhibition of IL6, COL1A1, and SPP1 with the help of their respective inhibitory drugs (**Table 1**) might have a regulatory influence on the immune system, and degradation of extracellular matrix pathways. In a nutshell, these data will provide new insight into the targeted therapy of LUAD patients with respect to the identified hub gene expression.

Discussion

The LUAD subtype of lung cancer is the most prevalent cancer around the globe [31]. In some countries, the death incidence due to LUAD is ranked highest relative to other causes [1]. The traditional methods that are being used for detecting LUAD have poor sensitivity [1]. Therefore, mostly LUAD patients are diagnosed at an advanced stage and have missed enough opportunities to get treatment. Moreover, the available therapeutic regimens for LUAD patients have failed to significantly improve the survival of patients [32], so it is urgently needed to study molecular mechanisms governing LUAD.

During the last decade, microarray technology has improved significantly [9]. Gene expression microarrays have proven to be the most reliable tools for analyzing the DEGs associated with the development and progression of LUAD [10, 11]. Many previous studies on LUAD have not integrated gene expression data across-platforms [12] to explore the molecular basis of LUAD. Therefore, the utility of cross-platform analysis will increase the reliability of the results. In our study, we retrieved GSE10072 from the GEO. Then, after comparative analysis between LUAD and control samples, a total of the top 100 DEGs were screened and subjected to protein interaction and top 6 hub genes identification analysis. Based on the degree

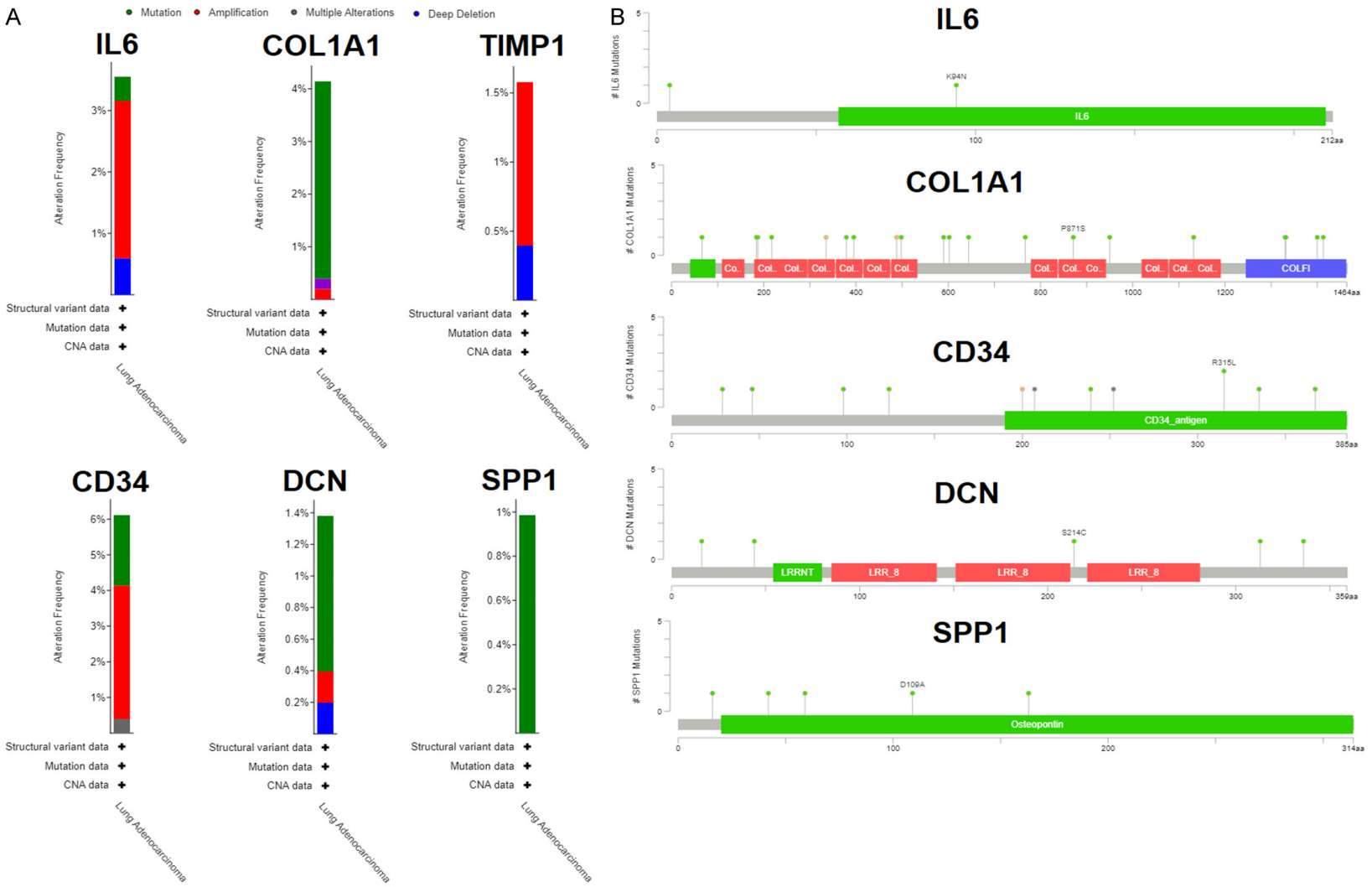


Figure 6. Evaluation of hub genes genomic alterations in the TCGA LUAD cohort via cBioPortal. (A) These graphs highlighted the percentages and types of genomic alterations in hub genes, and (B) These lollipops highlighted the amino acid change and their position across proteins encoded by hub genes. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1.

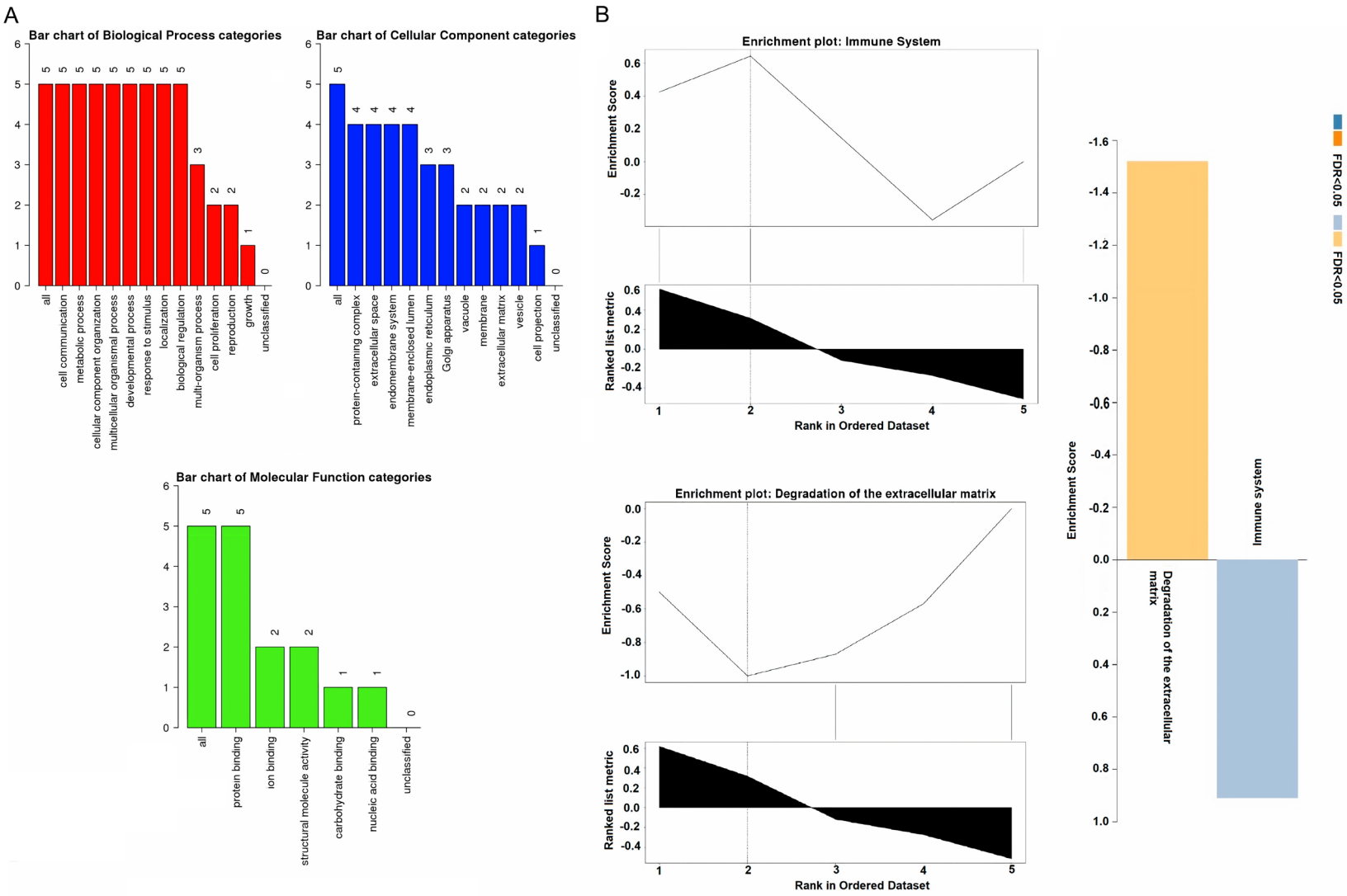


Figure 7. GSEA analysis results of 6 hub genes. (A) Hub genes associated BP, CC, and MF terms, and (B) Hub genes associated KEGG terms. A *p* value score of less than <0.05 was considered significant.

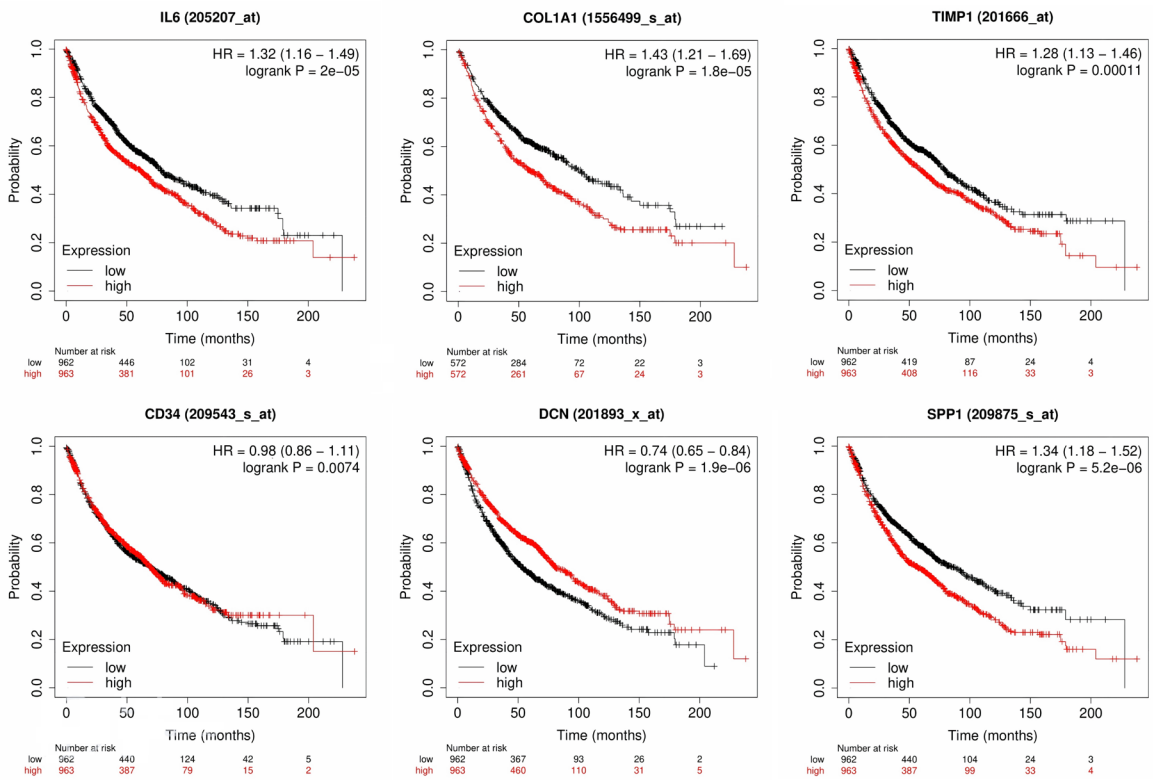


Figure 8. Prognostic significance (OS analysis) of the 6 hub genes across LUAD patients. A *p* value score of less than <0.05 was considered significant. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1, HR = Hazard ratio.

method, the identified hub genes across LUAD samples include IL6, COL1A1, TIMP1, CD34, DCN, and SPP1. Further GSEA analysis revealed that these hub genes were enriched in cell communication, metabolic process, and cellular component organization, etc., BP terms, protein containing complex, extracellular spaces, and endomembrane system, etc., MF terms, protein binding, ion binding, structural molecule activity, and carbohydrate binding, etc., CC terms, and immune system, and degradation of extracellular matrix pathway terms.

IL6 was earlier reported to be involved in immune responses, bone metabolism, arthritis, inflammation, neoplasia, reproduction, and aging [33]. The elevated level of IL6 is considered a reliable diagnostic factor among different types of cancers, including multiple myeloma, renal cell carcinoma, Hodgkin's lymphoma, bladder carcinoma, esophageal squamous cell carcinoma, ovarian cancer, and breast cancer [34-37]. The higher expression of IL6 is found to be linked with tumor aggressiveness, and poor survival in these cancers. Moreover, the higher expression of IL6 contributes to cancer metastasis by decreasing the cell adhesion ability of cancer cells (13-16). In contrast to the previous studies, we observed that IL6 expression was down-regulated and associated with favorable OS in LUAD patients.

COL1A1 encodes for the pro-α 1 chains of type I collagen and has three conservative domains known as von Willebrand factor type C (vWFC), collagen triple-helix repeat, and fibrillar collagen C-terminal domain (COLF) [38]. According to recent reports, the up-regulation of COL1A1 was found to be associated with a variety of solid tumors such as tumor of the breast, colon, cervix, hepatocellular carcinoma, and thyroid cancer [39-42]. Moreover, COL1A1 overexpression was also linked to the poor survival, recurrence, and metastasis of different cancers [43]. Just like previous reports, we also observed that COL1A1 expression was elevated and associated with unfavorable OS in LUAD patients via this study.

TIMP1 encodes for a 207 amino acid protein involved in the inhibition of the proteolytic activity of matrix metalloproteinases (MMPs) by

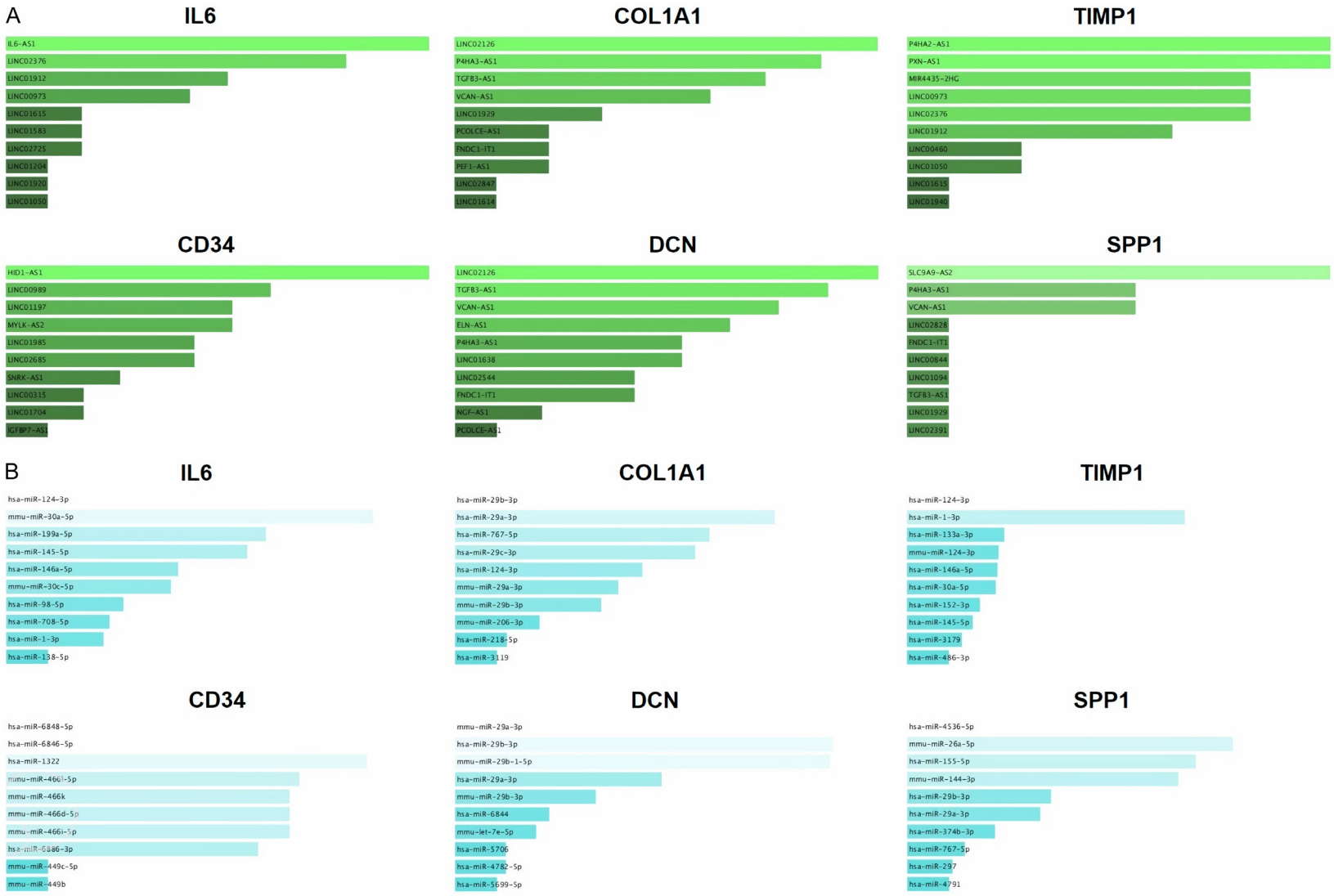


Figure 9. Prediction of the hub genes-associated lncRNAs and miRNAs via Enrichr. (A) Prediction of hub genes-associated lncRNAs, and (B) Prediction of hub genes-associated miRNAs. A *p* value score of less than <0.05 was considered significant. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, TIMP metallopeptidase inhibitor 1 = TIMP1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1.

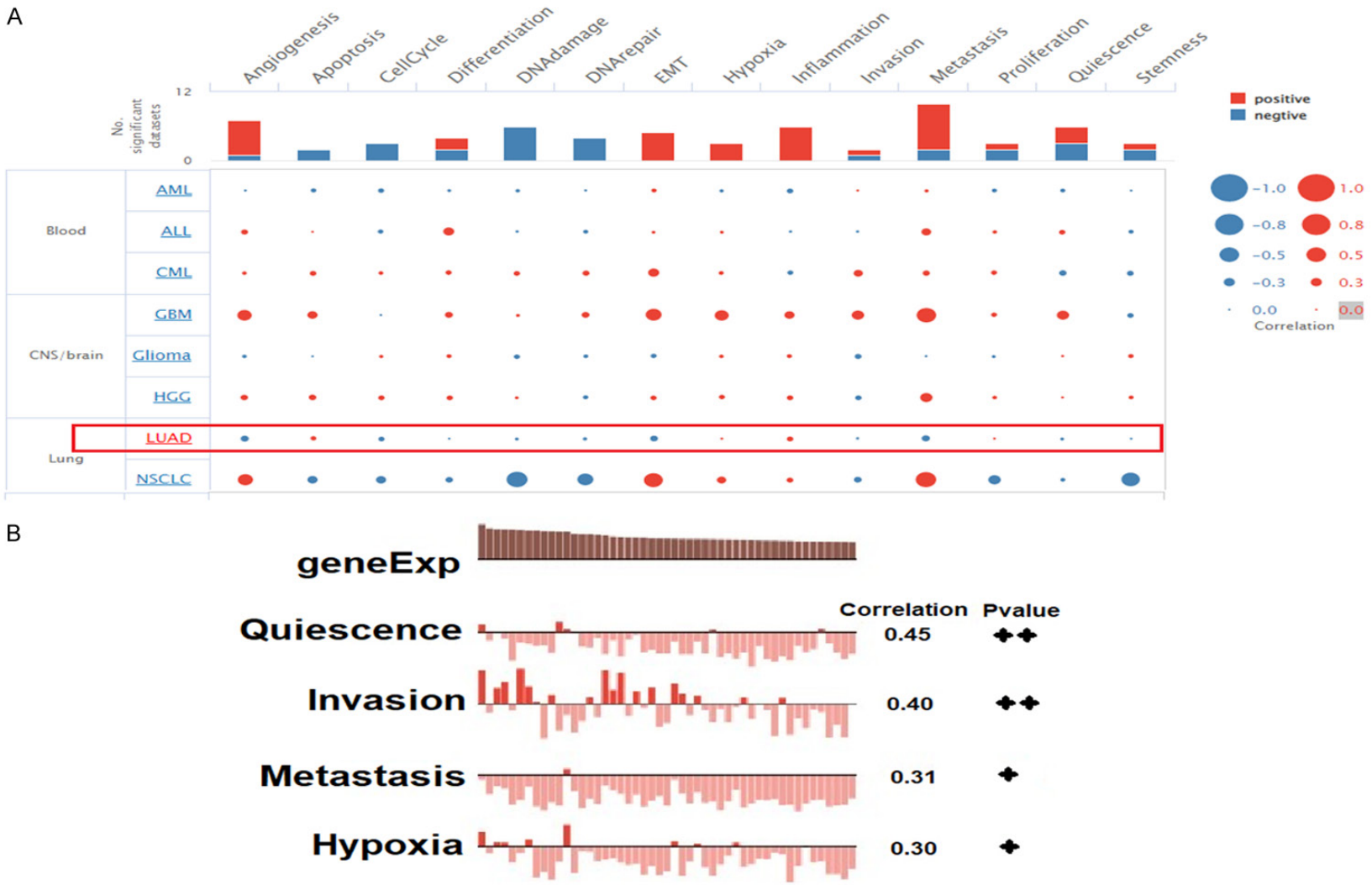


Figure 10. Exploring correlations among hub genes and different important states at single cell level in LUAD. (A) Correlation analysis of hub genes expression with 14 different states in LUAD, and (B) Important states showing significant correlations with hub genes. A *p* value <0.05 was consider as significant. EMT = Epithelial-mesenchymal transition.

Table 1. DGIdb-based hub genes associated drugs

Sr. No	Gene	Drug	Interaction	Mechanism	Interaction score
1	IL6	SILTUXIMAB	inhibitory	Interleukin-6 inhibitor	9.89
2	IL6	OLOKIZUMAB	inhibitory	Interleukin-6 inhibitor	9.89
3	IL6	CLAZAKIZUMAB	inhibitory	Interleukin-6 inhibitor	7.42
4	COL1A1	OCRIPLASMIN	inhibitory	Collagen hydrolytic enzyme	0.84
5	CD34	PUROMYCIN	----	----	3.86
6	CD34	PREDNISOLONE	----	----	1.24
7	DCN	SIROLIMUS	----	----	2.21
8	SPP1	ASK-8007	inhibitory	Osteopontin inhibitor	10.3
9	SPP1	CALCITONIN	----	----	3.43
10	SPP1	ALTEPLASE	----	----	1.08
11	SPP1	TACROLIMUS	----	----	0.21

Interaction score is based on the number of references and databases supporting to a particular interaction. Interleukin 6 = IL6, Collagen, type I, alpha 1 = COL1A1, CD34 molecule = CD34, Decorin = DCN, and Secreted Phosphoprotein 1 = SPP1.

forming noncovalent 1:1 stoichiometric complexes [44]. Recent studies revealed that the dysregulation of TIMP1 is related to unfavorable prognosis across different solid tumors, like gastric cancer [45], cutaneous melanoma [46], breast cancer [47], and papillary thyroid carcinoma [46]. Moreover, higher TIMP1 expression is associated with cancer stages, poor survival, and metastasis of liver cancer. Furthermore, it was also observed that TIMP1 expression was elevated significantly in the blood of gastric cancer patients [48] and patients with familial pancreatic cancer [49]. Just like previous reports, we also observed that TIMP1 expression was elevated and associated with unfavorable OS in LUAD patients.

CD34 gene encodes for a transmembrane phosphoglycoprotein protein [50]. This gene was originally considered as the molecular biomarker of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells [51]. Now, the role of down-regulated CD34 in diagnosing, measuring prognosis, and treatments of other solid tumors has been thoroughly discussed, such as in colon cancer, liver cancer, pancreatic cancer, breast cancer, ovarian cancer, and urothelial cancer [5, 52, 53]. Moreover, CD34 expression has also been shown to regulate angiogenesis in gliomas by accelerating the formation of new blood vessels [54, 55]. In our study, we observed that CD34 expression was down-regulated and associated with unfavorable OS in LUAD patients.

The DCN gene encodes for decorin protein, which is a key component of the extracellular matrix [56]. Previously, the published literature has shown significant down-regulation of DCN expression across different cancers and solid tumors such as breast, prostate, vascular, colon, and bladder cancers, myelomas, malignant peripherial nerve sheath, and liposarcomas, tumors [57-59]. Furthermore, the low expression of DCN was also linked with the poor survival duration of cancer patients [57-59]. Just like previous studies, in our study, we observed that DCN expression was elevated and associated with unfavorable OS in LUAD patients.

SPP1 is a secreted arginine glycine aspartic acid containing phosphorylated glycoprotein [60]. The hyper expression of SPP1 is notably related to the metastasis in gastric cancer and esophageal adenocarcinoma [61-63]. Moreover, previous studies also suggested the utility of highly expressed SPP1 as a molecular biomarker in numerous solid tumors, such as lung cancer, breast cancer, prostate cancer, and colon cancer [63-65]. Just like previous studies, in our study, we also observed that SPP1 expression was elevated and associated with unfavorable OS in LUAD patients.

Methylation and genetic alteration analyses of the hub genes showed that promoter hypermethylation was only linked with the lower expression of IL6, CD34, and DCN hub genes. DNA methylation is a reversible process; therefore, targeted therapies can help to control the expression of these genes in patients with LUAD. Moreover, hub genes were not found genetically altered in too many LUAD samples. Therefore, we speculate that genetic alterations do not participate in the dysregulation of

hub genes. Moreover, we also explored 10 experimentally validated lncRNAs and miRNAs in order to construct an lncRNA-miRNA-mRNA network in relation with hub genes that could help to understand the development of LUAD at the molecular level in more depth.

To understand the in-depth biological roles of the hub genes in LUAD development, we utilized the cancerSEA database to analyze their correlation with 14 different states in LUAD at the single cell level. Results shown that hub genes were significantly positively correlated with quiescence, invasion, metastasis, and hypoxia states in LUAD. To the best of our knowledge, this research is the first to collectively investigate such roles of identified hub genes in LUAD development. Finally, a total of 11 drugs having therapeutic potential against hub genes were identified using the DGIdb database. The reliable target hub genes of these drugs were IL6, COL1A1, and SPP1. Nevertheless, based on this knowledge of hub gene-drug interaction, we speculate that LUAD patients may benefit from these drugs during their treatment.

Conclusion

Through this study, a total of 6 hub genes (IL6, COL1A1, TIMP1, CD34, DCN, and SPP1) have been identified through an integrative bioinformatics approach. To further verify if these hub genes are associated with LUAD, we utilized OncoDB and GENT databases for the expression validation. Analysis results showed that 3 hub genes (IL6, CD34, and DCN) were significantly down-regulated and 3 hub genes (COL1A1, TIMP1, and SPP1) were significantly up-regulated in LUAD samples and cell lines relative to controls. Therefore, we believe that the identified hub genes are significantly involved in the development of LUAD and seem to be reliable biomarkers for diagnosis and prognosis purposes.

Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project number (RSPD2023R725) King Saud University, Riyadh, Saudi Arabia.

Disclosure of conflict of interest

None.

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