Multidisciplinary Cancer Investigation **Original Article**

October 2024, Volume 8, Issue 4

Differentially Expressed mRNAs in Gefitinib Resistance Human Lung Adenocarcinoma Cell Lines: A Bioinformatics Approach

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Submitted: 28 August 2024

Revised: 20 September 2024

Accepted: 05 November 2024

e-Published: 15 December 2024

Keywords:
Lung adenocarcinoma
Gefitinib resistance
mRNA expression profile
Immune infiltration

Lung adenocarcinoma (LUAD) is the most common histological subtype of non–small cell lung cancer. Differential gene expression profile of tumors is a crucial event influencing various cancer traits, such as resistance to therapies. We evaluated the mRNA profile, hub genes, and pathways in two gefitinib-resistance (GR) LUAD cell lines. Differentially expressed genes (DEGs) associated with acquired GR were identified from the gene expression profile GSE169513 (for PC9 cells) and GSE123066 (for HCC4006 cells). PPI networks of upregulated mRNAs were obtained based on the STRING database and Cytoscape software. R packages were conducted for enrichment pathway analysis. 7128 and 2812 GR-related DEGs were identified in PC9 and HCC4006 cells, respectively. GR-related genes influence cytokine signaling and extracellular matrix organization in PC9 and HCC4006 cells. The high-expressed hub genes were obtained (19 in PC9 and 16 in HCC4006 GR cells), in which SERPINE1 and CDH2 overlapped in both GR cell lines. SERPINE1 was also the overlapped hub gene in the transcription regulatory networks of both cell lines based on the TRRUST database. Significant correlations between the expression of hub genes and tumor infiltration of cancer-associated fibroblast based on the TIMER2.0 database. Overall, two drug resistance cell lines employed different global gene expression alterations. Targeting the upregulated hub genes could restore immune and mechanical abnormalities of the tumor microenvironment and may be a new approach for overcoming the GR in LUAD.

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INTRODUCTION

Lung cancer is one of the leading causes of cancer-dependent death. Coughing, shorted breath, repeated lung infection, chest pain, loss of appetite, fatigue, and weight loss are common symptoms of lung cancer. Small cell lung cancer (SCLC) and non–small cell lung cancer (NSCLC) are the two main histological groups of lung cancers. Lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and large cell carcinoma are classified in the NSCLC. LUAD and LUSC are more common types of lung cancer [1]. The therapeutic options for patients with lung cancer include surgery, radiotherapy, chemotherapy, immunotherapy, and targeted therapy. Gefitinib (Iressa) is an example of targeted therapy that inhibits epidermal growth factor receptor (EGFR)

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[DOI: 10.66224/mci.2024.701.1]

tyrosine kinase. However, reasons, such as a secondary mutation T790M in EGFR or changes in cancer cell transcriptome, cause gefitinib resistance in NSCLC [2]. Drug resistance is a main drawback of the targeted therapy approach. This is either hereditary or acquired during the cancer progression. Various studies confirmed that mutations and transcriptome changes account for most drug resistance in cancer [3]. The gene expression disturbance can result in alterations in the apoptosis and cell cycle, alteration in signaling pathways, impairment of DNA repair systems, and overexpression of drug efflux transporters [4]. For example, Li et al. showed that dysregulation of 4 hub genes, PI3, S100A8, AXL, and PNPLA4, were the most relevant genes to GR in PC9 LUAD cells, and these genes were mainly associated with cell cycle [5]. Gene expression profiles influence cellular functions and behaviors, making transcriptional perspectives critical for studying cancer. Understanding these molecular dynamics provides deeper insights into cancer biology [6, 7].

This study focused on transcriptional analyses to explore the molecular mechanisms driving gefitinib resistance in LUAD. This research utilized bioinformatics approaches to identify genes exhibiting differential expression between gefitinib-resistant (GR) and gefitinib-sensitive (GS) LUAD. Resistance-associated genes were pinpointed by comparing microarray data from drug-resistant and drug-sensitive cancer groups. Specifically, the gene expression datasets GSE169513 and GSE123066 were analyzed to determine differentially expressed genes (DEGs) in GR GS PC9 and HCC4006 cell lines, respectively. The study aimed to uncover key upregulated genes implicated in GR in LUAD, as targeting these upregulated genes may help reverse resistance to gefitinib. Furthermore, the relationship between key genes and cancer-associated fibroblasts (CAFs) or macrophage infiltration within the tumor microenvironment (TME) was investigated.

METHODS

Datasets and data processing

The GSE169513 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE169513) and GSE123066 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE123066) datasets containing the microarray data of acquired GR and GS cells from the GEO database were downloaded for PC9 and HCC4006 cells, respectively. We included three biological replicates of datasets for each group (GR or GS) in our analysis (Table 1). DEGs between drug resistance and control groups (i.e., GS cells) were identified separately for each cell line via the GEO2R online tool and R program (version 4.3.2.). Principal component analyses (PCA) plot was used to evaluate the variances in gene expression levels of all replicates in GSE datasets. Whenever required, log2 transformations were applied to reduce data variability. Limma R package [8] was used for normalization and DEG identification in R software. The genes with adjusted P value threshold of 0.05 and $|\log2FC| \geq 0.5$ were selected as DEGs. Then, the hub DEGs of the datasets were investigated and compared. Figure 1 shows the flow chart of our methodology.

To evaluate the hub genes in LUAD patients, LUAD data were obtained from The Cancer Genome Atlas (TCGA, http://portal.gdc.cancer.gov/). RNA-seq data quality control, normalization, and batch effect correction were performed using the TCGA biolinks

Table 1. Information on the datasets used in this study.

Dataset	Characteristic	Sample	Control
GSE169513	PC9 LUAD cell line	GR cells (3 biological replicates)	GS cells (3 biological replicates)
GSE123066	HCC4006 LUAD cell line	GR cells (3 biological replicates)	GS cells (3 biological replicates)
TCGA-LUAD	American Indian or Alaska native (0.2%) Asian (1.4%) Black or African American (9.1%) White (67%) Unknow/not reported (22%)	541 LUAD tumor tissues	59 matched adjacent normal tissues

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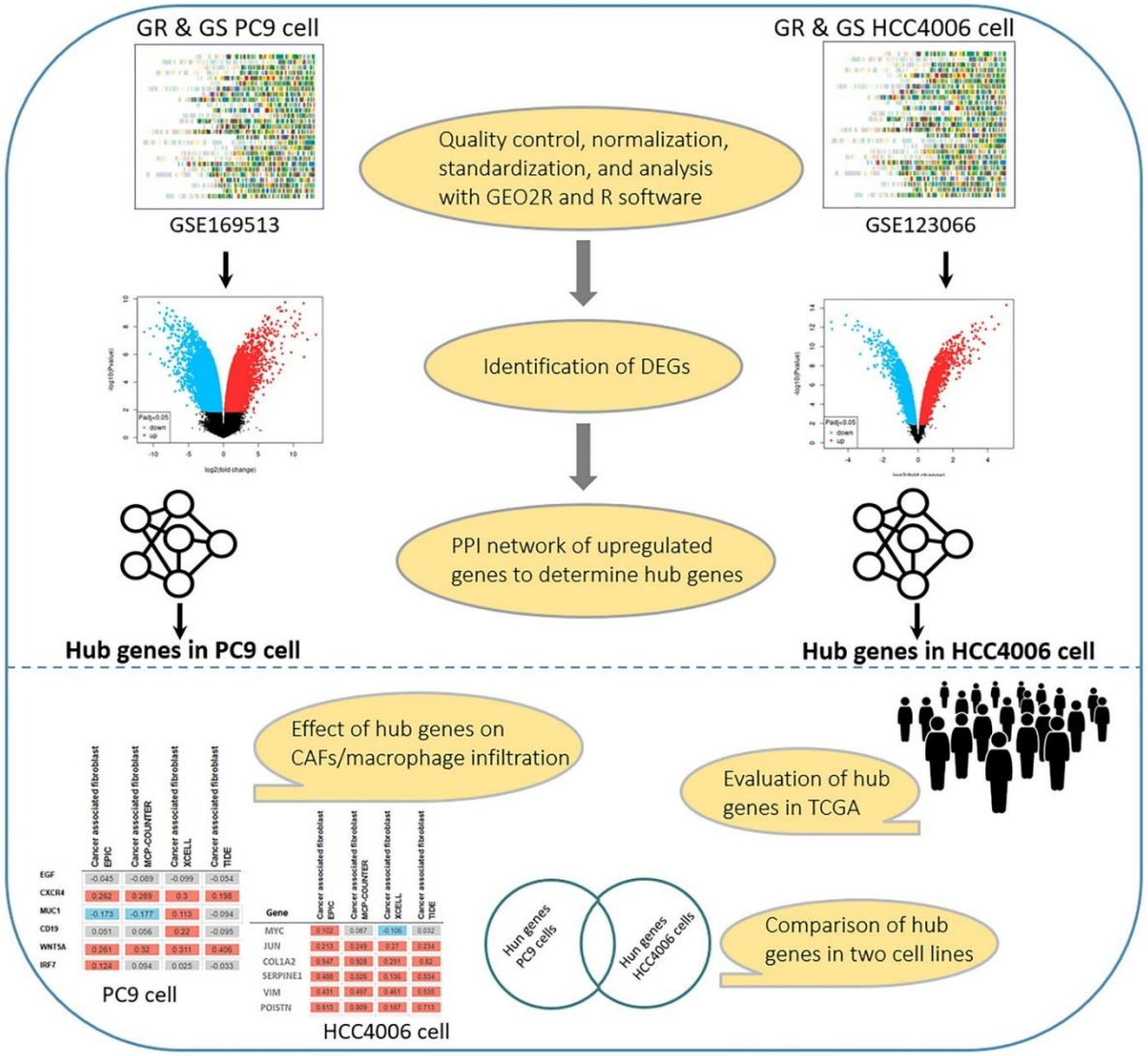


Figure 1. Flowchart of bioinformatics analysis included data collection, processing, analysis, and investigation. GR: gefitinib-resistant. GS: gefitinib-sensitive. DEGs: differentially expressed genes, PPI: protein-protein interaction network, CAFs: cancer-associated fibroblast, and TCGA: The Cancer Genome Atlas.

[8], edgeR [9], and limma R [9] packages, respectively. The TCGA-LUAD data (Nov 2023) included 541 cases of clinical tumor samples and 59 matching adjacent normal tissues as the control group (Table 1). A Limma package extracted DEGs from the tumor and standard samples from the TCGA database. The cut-off was adjusted P value ≤ 0.05 and |log2FC| ≥ 0.5.

Determining hub genes

For the construction of a protein–protein interaction (PPI) network of DEGs, STRING database (https://string-db.org) was used. Interactions with scores > 0.4 were recognized as statistically significant. The results were visualized with Cytoscape (version 3.10.0) software. To select the hub genes, CytoHubba, which is a plug-in of Cytoscape, was employed to identify the hub genes. The degree algorithm in CytoHubba was used to evaluate and select hub genes. The degree algorithm is the most basic and popular network centrality measure to identify essential nodes (i.e., genes) participating in the PPI network. It has been widely used to analyze biological networks in which genes with more neighbors or interactions have a higher value and influence in the network [10].

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Pathway enrichment analysis

R packages (org.Hs.eg.db [11] and enrichR [12]) were conducted for KEGG_2021 and Reactome_2022 analyses of upregulated genes in GRLUAD PC9 and HCC4006 cells. plotEnrich package [12] was used for the visualization of the results (adjusted *P* value ≤ 0.05).

Correlation between the expression of hub genes and CAFs/macrophage infiltration

TIMER2.0 database (http://timer.cistrome.org/) was applied to evaluate the correlation of hub gene expression with immune infiltration level in LUAD. Different types of methods (EPIC, quanTIseq, XCELL, CIBERSORT, QUANTISEQ, TIDE, and MCP-COUNTER) were performed for the association analysis.

Prediction of TFs

To identify TFs with possible effects on differential gene expression between drug resistance and sensitive groups, we used the transcriptional Regulatory Relationship Unraveled by Sentence Based Text Mining (TRRUST) database to predict TFs that regulate hub genes (adjusted *P* value ≤ 0.05). Then, the overlapping of the detected TFs with dysregulated mRNA in GR LUAD cells was investigated using the Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/). A Venn diagram shows the similarities and differences between two or more groups.

RESULTS

Identification of DEGs

A total of 7128 DEGs (|log2FC| ≥ 0.5 and adjusted *P* value ≤ 0.05) were found in GSE169513 datasets. 4282 genes were upregulated, and 2846 were downregulated in GR compared to GS LUAD cells (Figure 2A and B). A total of 2812 DEGs (|log2FC| ≥ 0.5 and adjusted *P* value ≤ 0.05) were found in GSE123066 (for HCC4006 cells). 1317 DEGs were upregulated, and 1495 DEGs were downregulated in GR compared to GS LUAD cells (Figure 2C and D). After the integration of the DEGs (excluding DEGs that were upregulated in one haplotype and downregulated in another haplotype or merging different haplotypes of some other DEGs), the 500 top upregulated mRNAs (based on log2FC) were selected in GR compared to GS LUAD cells in each dataset for further analysis. The overexpressed hub genes can be silenced by techniques such as siRNAs or CRISPRi to overcome drug resistance. Figure 2 depicts fold

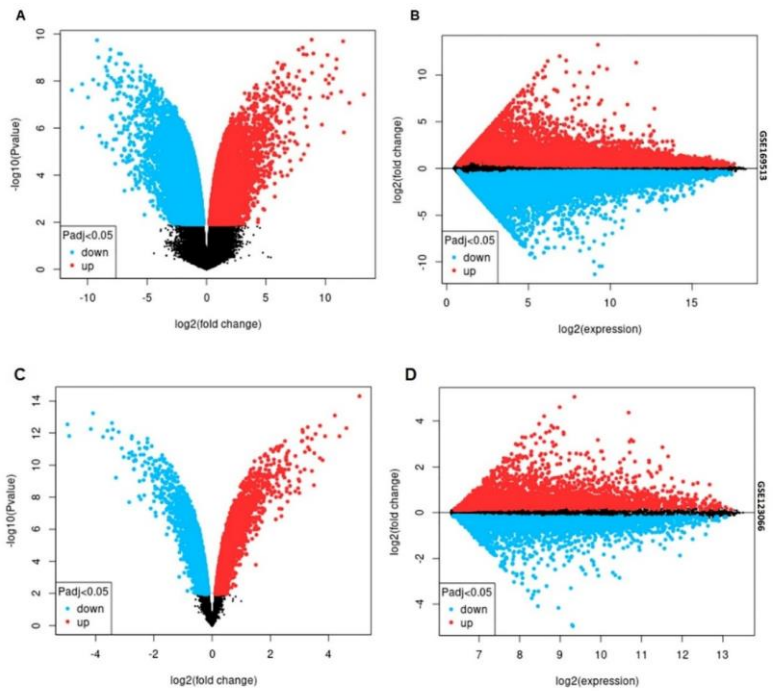


Figure 2. Volcano plot (A and C) and mean-difference plot (B and D) of GSE169513 and GSE123066 dataset, respectively (GR vs GS LUAD PC9 and HCC4006 cells).

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changes of gene expression in PC9 cells more than 2 times in HCC4006 cells. Therefore, in PC9 cells, the top 500 overexpressed genes had log2FC > 2.8, but the top 500 DEGs in HCC4006 cells had log2FC > 0.8.

Approximately 8193 mRNAs were dysregulated in LUAD according to TCGA ($|log2FC| \geq 0.5$ and adjusted P value ≤ 0.05). In this study, 3773 mRNAs were upregulated, and 4420 mRNAs were downregulated in tumors compared to normal tissues.

PPI network construction of GR-related genes, selection of hub genes, and pathway enrichment analysis

The PPI network for the GR-related upregulated genes was constructed using STRING. The CytoHubba (Degree algorithm) in Cytoscape was used to analyze the STRING data. According to the Degree method, upregulated hub genes (19 in PC9 and 16 in HCC4006 GR cells) were identified for further analysis.

As depicted in Table 2, the expression level of CXCR4, MUC1, CCL3, WNT5A, SERPINE1, and GATA4 genes only differed between GR and GS PC9 cells, not between tumor and normal tissues. Unlike the TCGA database for tumor and normal tissues, the expression level of ALDH1A1, CD40, EGR1, GNG7, GLI1, BMP2, IGF2, and IL6R showed upregulation in GR PC9 cells (Table 2). MYC, SERPINE1, LOX, COL6A1, and SPARC genes were upregulated in GR HCC4006 cells with no changes in the TCGA database for LUAD tumors and normal tissues. JUN, VIM, CXCL12, FGF2, CAV1, and ITGA5 showed upregulation in GR HCC4006 cells and downregulation in the TCGA database for tumor and normal tissues.

In Reactome-2022 and KEGG-2021 analyses, the most significant cancer-related enrichment pathway for the top GR-related upregulated genes was cytokine signaling in PC9 cells (Figure 3B). Extracellular matrix (ECM) organization was the most significant pathway for GR-related genes in HCC4006 cells (Figure 4B). This was followed by a KEGG-2021 analysis, which identified focal adhesion (a type of cell-matrix adhesion) as the most significant pathway in GR HCC4006 cells. In this study, Reactome-2022 analysis covered a higher number of genes in pathways compared to KEGG-2021 (Supplementary Figure 1), so only the result of Reactome-2022 analysis was shown in Figures 3 and 4. Moreover, in the human genome, Reactome classifications provide the best enrichment performance [13].

Overlapped hub genes in two cell lines

Overexpressed SERPINE1 and CDH2 genes can be predictive biomarkers for PC9 and HCC4006 GR cells. The SERPINE1 gene showed upregulation more than 16 times in GR than in GS cell lines (Table 2). The Kaplan-Meier survival analysis showed the prognostic value of the SERPINE1 gene based on the Gene Expression Profiling Interactive Analysis (GEPIA) database. Overall survival time of patients with high SERPINE1 expression levels was significantly shorter than that of patients with low expression (Figure 5B). Moreover, based on the GEPIA database and Pearson correlation (Figure 6), SERPINE1 expression was significantly correlated with the expression of EGR1, BMP2, JUN, CXCL12, and CAV1 ($P < 0.01$).

Hub genes influence the tumor's immune microenvironment.

Overexpression of most hub genes positively correlates with recruiting CAFs and macrophages to the TME (Figure 7). Compared to the PC9 hub genes, the ECM-related hub genes have higher correlations with CAFs/macrophage infiltration (specifically CAFs). CXCR4, CD40, CCL3, SERPINE1, VIM, CXCL12, and LOX showed a higher frequency of positive correlation with both CAFs and macrophage infiltrations to TME. As depicted in Figure 7, various types of immune infiltration tests showed high frequencies of positive correlation between hub genes and CAFs in LUAD (Supplementary Figures 2-5).

Possible regulators of the hub genes

Nine TFs that can regulate the hub gene expression were identified based on the TRRUST database (Figure 8A) in PC9 cells. Five TFs (STAT1, HEY1, SMAD4, YY1, PPARA) were upregulated and four ones (MYC, ETS1, BRCA1, and E2F1) were downregulated in GR PC9. Because four hub genes named EGR1, IRF7, GLI1, and GATA4 were also TFs, we identified their dysregulated targets in GR LUAD PC9 cells (Figure 8B). The EGR1 with logFC

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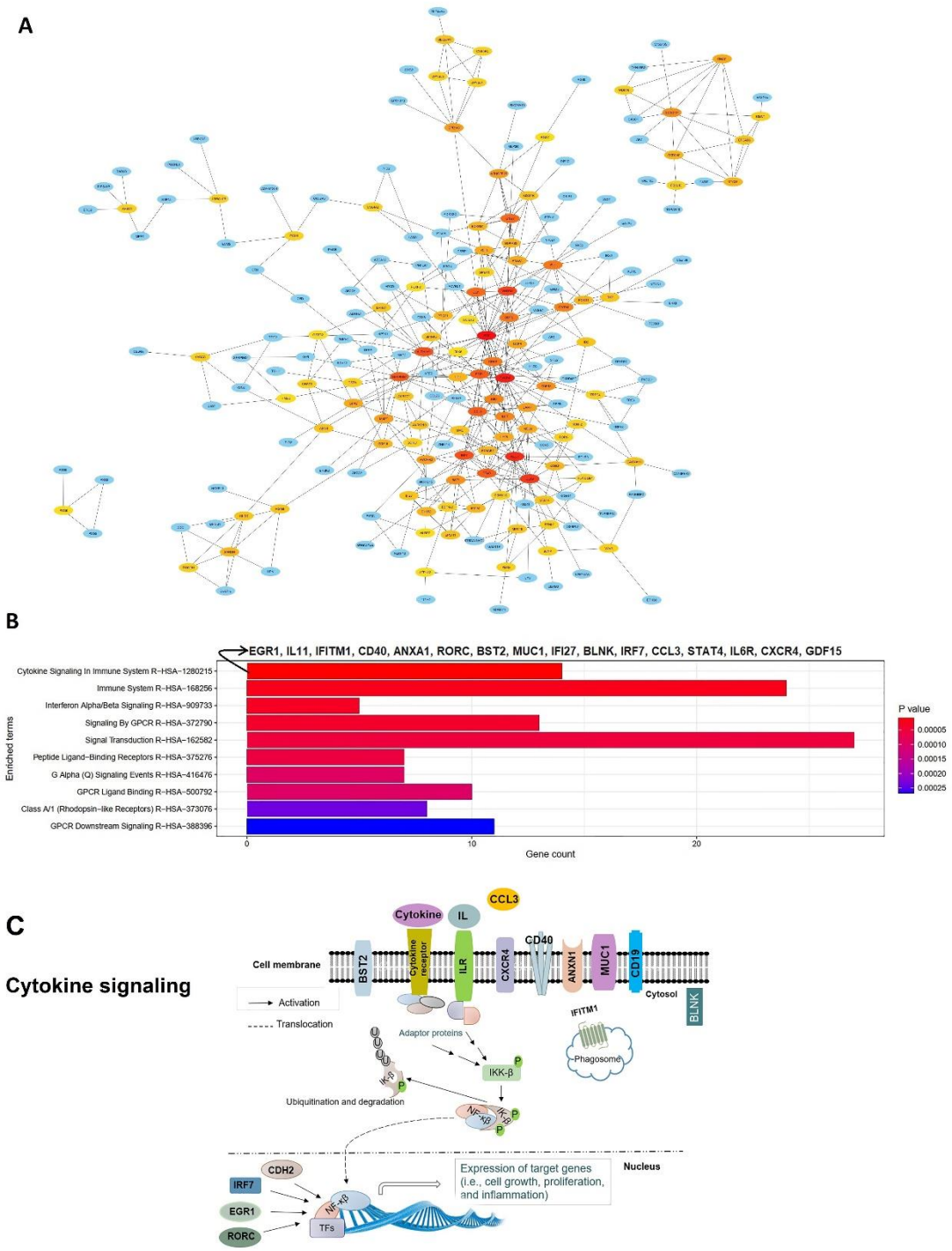


Figure 3. PPI network for the GR-related upregulated genes of PC9 cells in the CytoHubba (Degree algorithm) of Cytoscape, genes with more interactions are depicted with the high intensity of the red color (A), significant pathways related to upregulated mRNAs (B), and cytokine signaling pathway included GR related upregulated genes (C).

= 4.11 had the highest dysregulated targets (24 overexpressed and 19 low-expressed). ISL1 (as a hub gene) is also a TF; however, neither its targets nor its TFs are among the DEGs.

In HCC4006 cells, fifteen TFs of hub genes were among the DEGs. Twelve TFs (REAL, MYB, PTTG1, STAT3, TWIST2, AR, SNAI2, ZEB2, HIF1A, SMAD3, SMAD4, and E2F1) were

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Table 2. Identified hub genes in PC9 and HCC4006 GR cells and their characteristics.

Gene symbol	Gene name	Score (Degree method)	logFC (GR compared to GS)	logFC TCGA (tumor compared to normal tissues)	Selected study notes about the gene
PC9 cells					
EGF	Epidermal Growth Factor	28	3.25	1.49	EGF is a ligand of EGFR, which is the target of gefitinib [2].
CXCR4	C-X-C Motif Chemokine Receptor 4	22	3.64	0	Overexpression of CXCR4 promotes cisplatin resistance in NSCLC [14]. CXCR4 upregulation results in paclitaxel resistance in breast cancer [15]. Upregulation of CXCR4 is involved in drug-resistant NSCLC [16].
MUC1	Mucin 1	18	3.33	0	MUC1-mediated paclitaxel-resistance in lung cancer cells [17].
CD19	CD19 Molecule	16	3.21	1.86	CD19 was introduced as an indicator of immune disorders within TME [18].
WNT5A	Wnt Family Member 5A	15	3.48	0	WNT5A belongs to the WNT ligand family, and it was reported that WNT5A promotes epithelial-to-mesenchymal transition (EMT) in NSCLC, which is required for cancer metastasis [19].
IRF7	Interferon Regulatory Factor 7	14	3.15	1.01	Its overexpression in lung cancer cells was associated with resistance to virus infection [20].
ALDH1A1	Aldehyde Dehydrogenase 1 Family Member A1	14	7.23	-2.06	Overexpression of ALDH1A1 reflects the advanced stages of LUAD, and it is believed that a combination of anti-ALDH1A1 therapy and chemotherapy could overcome the ALDH1A1-induced drug resistance [21]. ALDH1A1 was reported as a predictive biomarker for erlotinib resistance, and it was shown that inhibition of ALDH1A1 overcame erlotinib resistance in LUAD [22].
CD40	CD40 Molecule	13	7.6	-0.74	CD40 might indicate advanced LUAD and its expression is significantly associated with better survival. Moreover, CD40 is an immune-related membrane receptor and might have a role in immune modulation within TME [23].
CCL3	C-C Motif Chemokine Ligand 3	13	5.51	0	Overexpression of CCL3 stimulates the secretion of IL-6 by TME cells in NSCLC [18].
EGR1	Early Growth Response 1	13	4.11	-1.9	Translocation of EGR1 (as a TF) to the nucleus was suppressed in resistant NSCLC [24].
SERPINE1	Serpin Family E Member 1	13	4.86	0	SERPINE1 is an NSCLC prognosis marker and a metastasis promoter in most cancers [25, 26]. SERPINE1 plays a role in the development of paclitaxel resistance in triple‐negative breast cancer, and its silencing led to the reversing of resistance [27].
GNG7	G Protein Subunit Gamma 7	12	3.74	-1.4	GNG7 is regarded as a tumor suppressor in lung cancer [28].

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GLI1	GLI Family Zinc Finger 1	12	3.66	-0.4	Overexpressed GLI1 was associated with platinum-based cancer drugs and EGFR-tyrosine kinase inhibitor resistance in LUAD [29].
ISL1	ISL LIM Homeobox 1	11	3.15	1.17	Overexpression of ISL1 promoted metastasis of gastric cancer cells [30].
BMP2	Bone Morphogenetic Protein 2	11	3.67	-2.24	BMP2 promotes cell migration and invasiveness in vitro and in vivo [31]. Overexpression of BMP2 correlates with metastasis in LUAD and inhibition of BMP2 receptor-sensitized lung cancer cells to tumor necrosis factor (TNF)-related apoptosis-inducing ligand [32].
CDH2	Cadherin 2	11	4.92	1.21	Overexpressed CDH2 can have a role in GR in NSCLC [33].
IGF2	Insulin Like Growth Factor 2	10	7.11	-0.88	IGF2 is a clinically relevant mechanism of osimertinib resistance in lung cancer [34].
GATA4	GATA Binding Protein 4	10	4.6	0	GATA4 is a TF whose expression specifically increased in GR compared to sensitivity. GATA4 is a tumor suppressor in lung cancer [35, 36].
IL6R	Interleukin 6 Receptor	10	3.82	-1.03	Inhibition of IL6 increased the sensitivity to gefitinib in NSCLC [37].
HCC4006 cells					
MYC	MYC Proto-Oncogene	70	1.25	0	Overexpressed MYC induces therapy resistance in multiple cancer types [38].
JUN	Jun Proto-Oncogene	49	1.64	-1.2	GR cells acquired an increased expression and activation of JUN in NSCLC [39].
COL1A2	Collagen type I alpha 2	39	3.09	1.17	Increased expression of COL1A2 was associated with drug resistance in ovarian cancer cell lines [40].
SERPINE1	Serpin Family E Member 1	39	4.96	0	-
VIM	Vimentin	38	1.87	-1.37	A key marker of EMT and involved in EMT-mediated multidrug resistance [41].
POSTN	Periostin	37	1.22	1.55	POSTN is involved in EMT and drug resistance in NSCLC [42].
CDH2	Cadherin 2	37	1.94	1.21	-
CXCL12	C-X-C Motif Chemokine Ligand 12	37	1.09	-1.65	The interaction between CXCL12 and CXCR4 causes tumor cells to form metastatic tumors [16].
COL5A1	Collagen type V alpha 1	36	1.3	1.73	COL5A1 may contribute to the metastasis of LUAD [43].
FGF2	Fibroblast Growth Factor 2	36	0.85	-2.5	FGF2 is involved in resistance in various cancer types [44].
LOX	Lysyl Oxidases	35	1.91	0	LOX led to resistance to chemotherapy through collagen stabilization in lung cancer [45].
CAV1	Caveolin-1	35	1.63	-4.1	Pumping out anti-tumor agents through membrane transporters leads to drug resistance in lung cancer [46].
COL6A1	Collagen type VI alpha 1	35	0.94	0	COL6A1 enhances lung cancer cell motility and metastasis [47].
THBS2	Thrombospondin 2	31	1.01	2.48	THBS2 is highly secreted via aggressive LUAD tumors and is associated with drug resistance [48].
SPARC	Secreted Protein Acidic and Rich in Cysteine	31	1.69	0	Overexpression of SPARC-induced EMT [49].
ITGA5	Integrin α5	31	1.44	-0.98	ITGA5 promotes cisplatin resistance in lung cancer [50].

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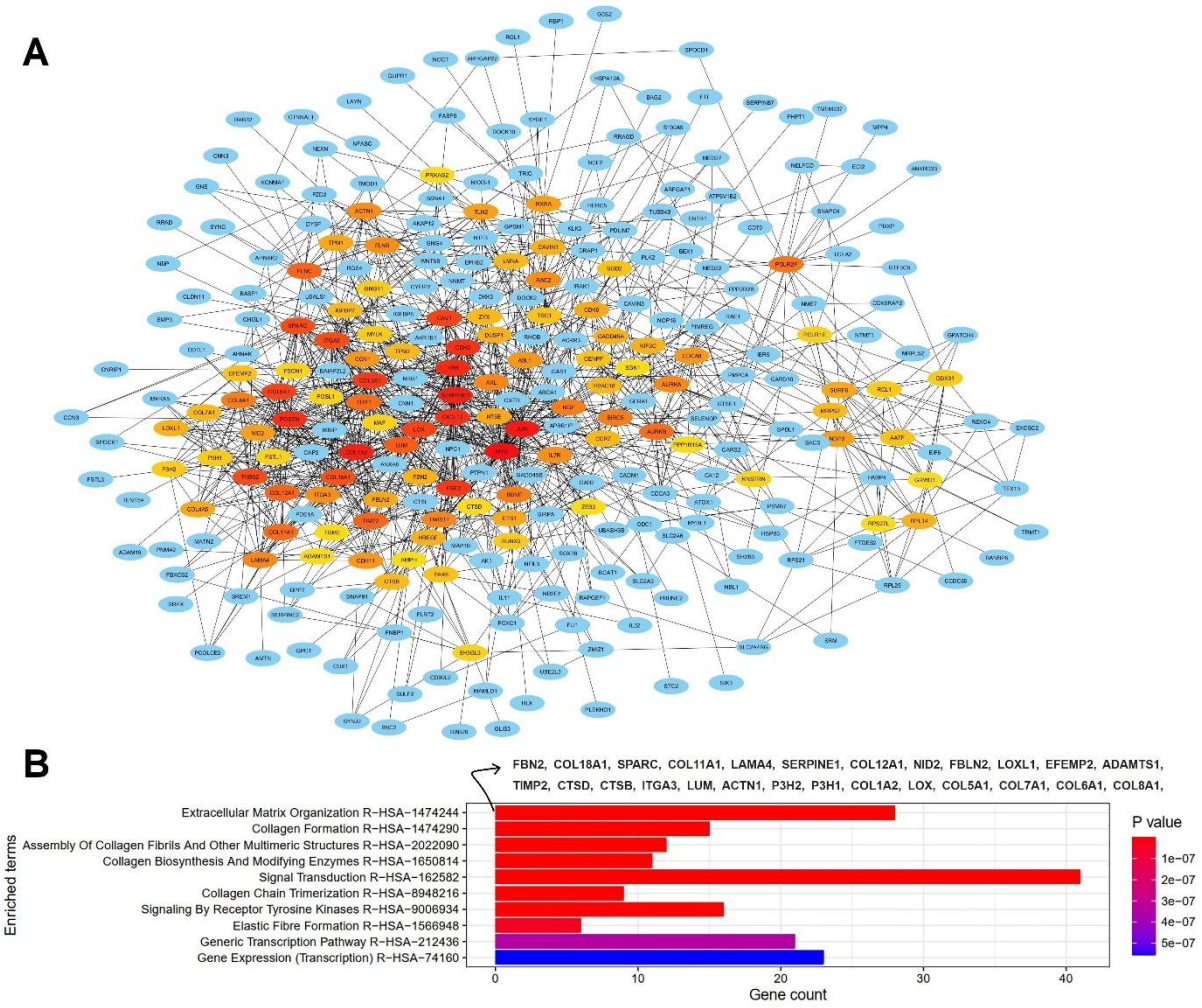


Figure 4. PPI network for the GR-related upregulated genes of HCC4006 cells in the CytoHubba (Degree algorithm) of Cytoscape, genes with more interactions are depicted with the high intensity of the red color (A), significant pathways related to upregulated mRNAs (B).

upregulated and three ones (ESR1, PPARG, and YY1) were downregulated in GR. MYC and JUN also act as TFs, so we identified their dysregulated targets in GR LUAD HCC4006 cells (Figure 8D). The highest number of dysregulated TFs influenced MYC and SERPINE1. Several TFs named RELA, PPARG, E2F1, SMAD3, SMAD4, and ESR1 target MYC and SERPINE1 genes. SERPINE1 was the overlapped hub gene in the transcription regulatory networks of both cell lines.

DISCUSSION

Identification of GR-related genes and signaling pathways in LUAD cell lines could help find an approach to overcome the GR. Cytokine signaling was the most significant enriched pathway of DEGs in PC9/GR cells. This result followed a previous study by Mao et al., who reported that cytokine signaling pathways are closely related to GR in LUAD patients [51]. The cytokine-regulated network is one of the dysregulated pathways in almost all cancers. Host-derived and tumor-derived cytokines can promote tumor growth and resistance to therapy [52]. In this study, CXCR4, MUC1, and CCL3 hub genes are related to cytokine signaling pathways (Figure 3). CXCR4 is a receptor for CXCL cytokines, and the tumorigenesis effect of CXCR4/CXCLs has been reported [37]. It was shown that inhibition of IL6 can re-sensitize multiple drug-resistant tumor cells. One reported mechanism for IL6 is the induction of cancer

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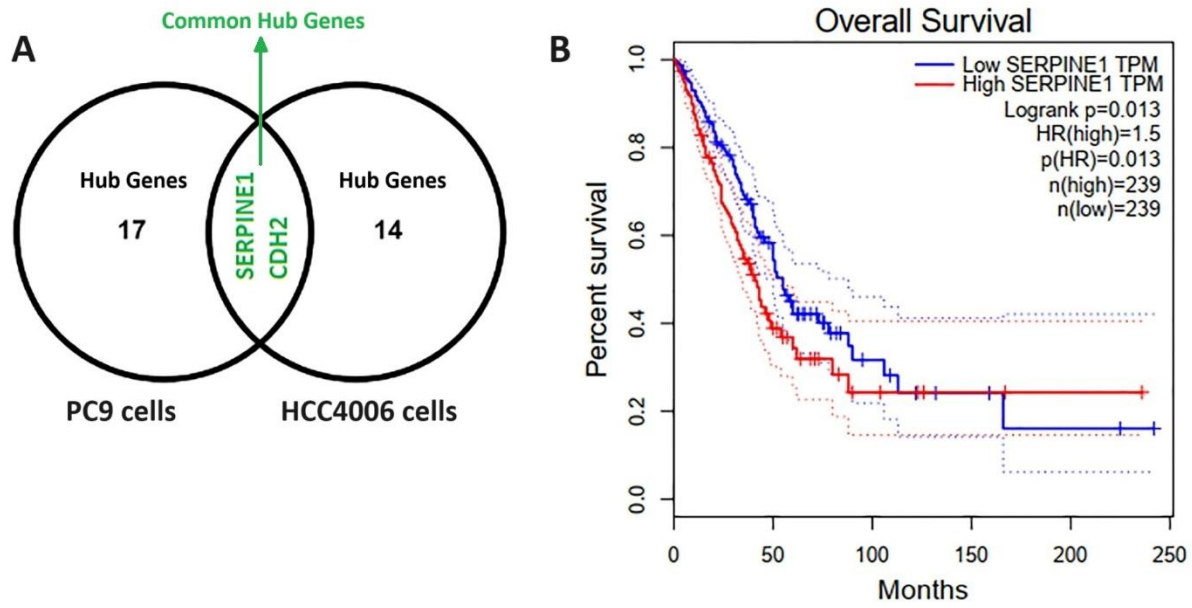


Figure 5. SERPINE1 as a predictive biomarker in both PC9 and HCC4006 GR cells. SERPINE1 and CDH2 were intersected hub genes of PC9 and HCC4006 GR cells according to Venn diagram (A). The significant association between SERPINE1 and overall survival in LUAD. The value of $P < 0.05$ was considered significant (B).

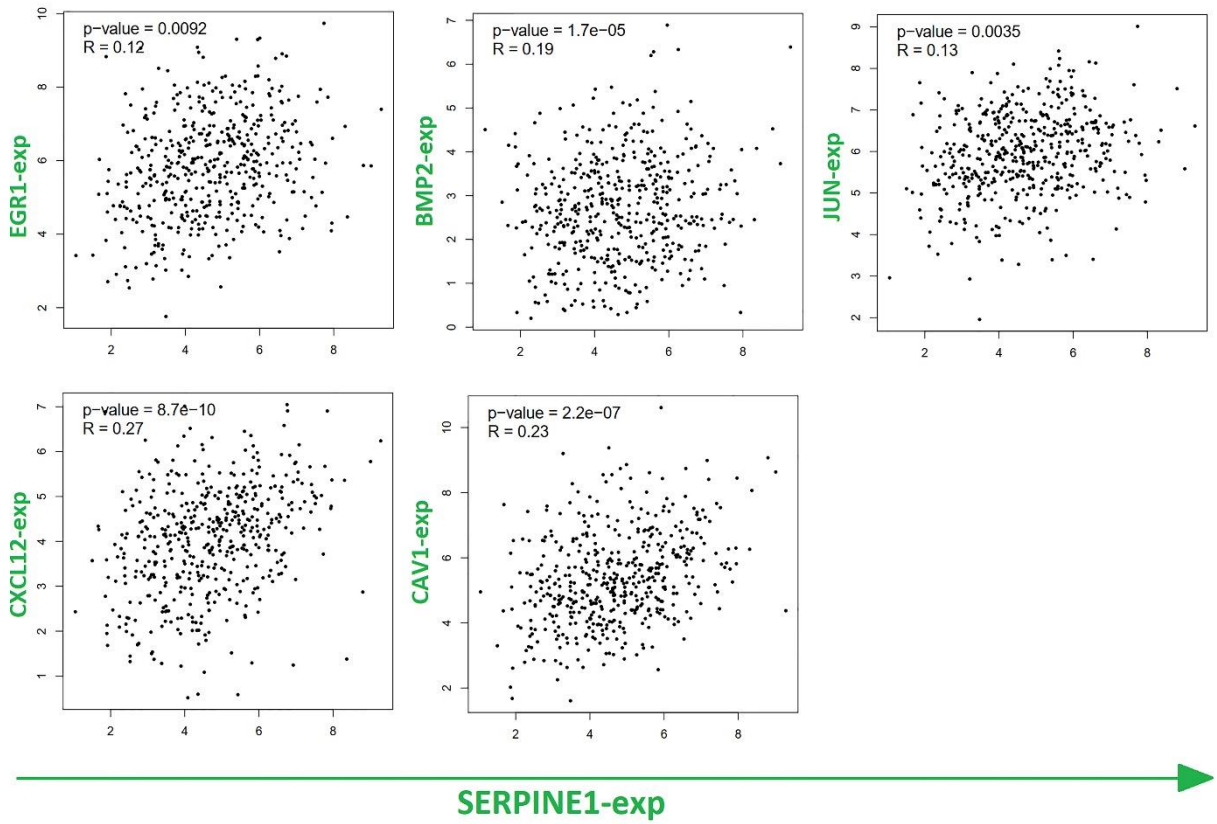


Figure 6. The expression of SERPINE1 has correlation with EGR1, BMP2, JUN, CXCL12, and CAV1 ($P < 0.01$).

stem-like phenotype leading to apoptosis escapes of cancer cells [37]. Accordingly, the receptor of IL6,

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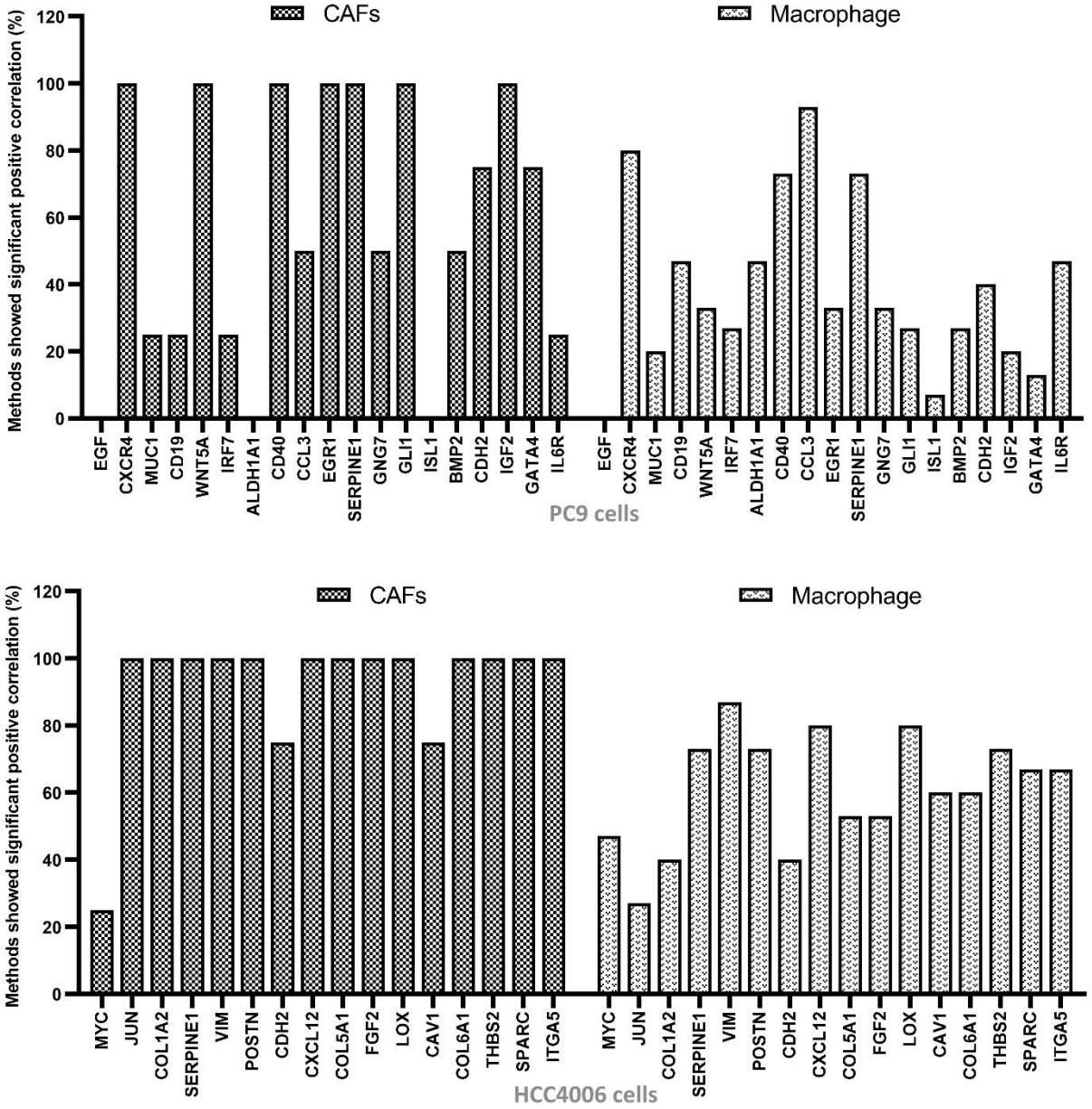


Figure 7. Correlation of hub genes (specifically CXCR4, CD40, SERPINE1, VIM, CXCL12, and LOX) expression with CAFs/macrophage infiltration. PC9 (A) and HCC4006 GR cells (B).

IL6R, was among the determined hub genes in PC9 cells.

GR-related genes in HCC4006 cells influence the TME through the modulation of ECM. Cancer cells are surrounded by an ECM, which is constructed from three major classes of macromolecules, including glycosaminoglycans (such as hyaluronan), fibrous proteins (such as collagen), and glycoproteins (such as laminin). ECM is an essential TME component and influences tumor progression and its response toward treatments [16]. The importance of TME in cancer progression and therapy outcomes has been well-established in numerous studies. TME includes cellular (such as cancer cells, stromal cells, tissue cells, macrophages, fibroblasts, etc.) and acellular (collagen, laminin, hyaluronan, growth factors, cytokines, small molecules, etc.) components that have interconnection with each other [16]. Each cell

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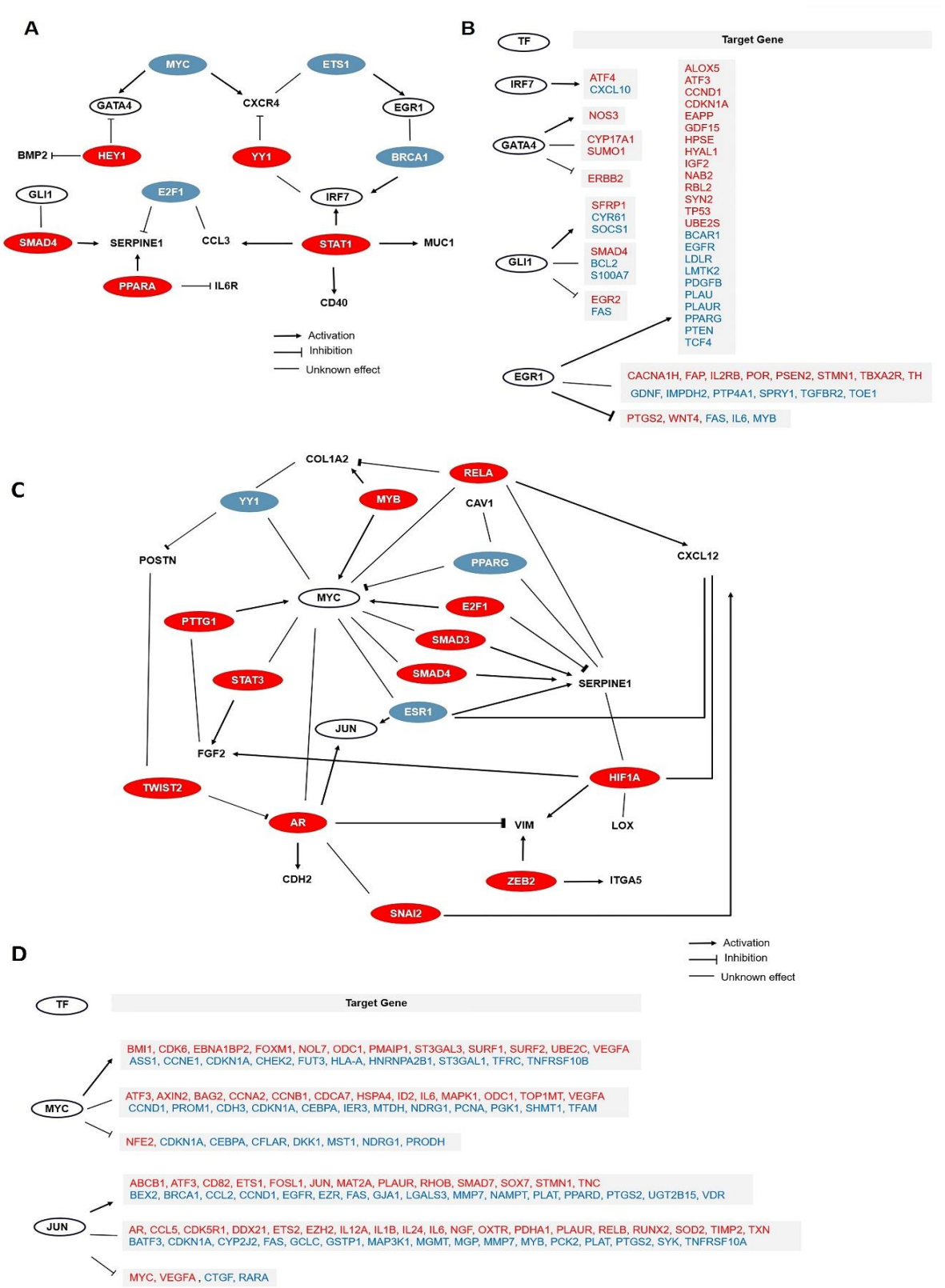


Figure 8. The expression regulatory network in GSE169513 and GSE123066 is based on the TRRUST database. Dysregulated TFs may influence the expression of the hub genes (written in black) determined in PC9 cells. Four hub genes (EGR1, IRF7, GLI1, and GATA4) act also as TFs (A). Target genes that can be regulated by hub genes (B). Dysregulated TFs may influence the expression of the hub genes (written in black) determined in HCC4006 cells. MYC and JUN also act as TFs (C). Target genes that can be regulated by MYC and JUN (D). Upregulated TFs are marked in red, and downregulated TFs are marked in blue (in A and C). Upregulated genes were written in red and downregulated in blue (in B and D). TFs are shown inside the circle. Although some target genes receive activator effects from TF/TFs, their expression decreases or vice versa; this suggests that there have been many other influencing factors on gene expression in both cell lines.

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modification leads to mechanical abnormalities in TME and changes in mechanosensitive or integrin signaling pathways [17]. It was reported that cancer cell adhesion to ECM within TME can protect them from chemotherapy-induced death [18] because tumor cell adhesion to ECM induces activation of integrin signaling and subsequent cell cycle arrest, drug efflux, and a phenotype switching of cancer cells to EMT or cancer stem cells [19].

Overexpressed SERPINE1 and CDH2 genes were overlapped hub genes for both GR cell lines. This result was reinforced by the study of Wang et al., who reported that SERPINE1 had higher expression in the GR PC9 cell line than in the GS PC9 line [53]. Moreover, Song et al. showed that knockdown of CDH2 could significantly increase sensitivity to gefitinib in NSCLC cell lines [54]. SERPINE1 encodes a serine peptidase inhibitor (also named plasminogen activator inhibitor-1) and is an essential regulator of ECM remodeling. SERPINE1 is a highly expressed gene in several cancers associated with tumors spreading to surrounding normal tissue. SERPINE1 is reported as a novel therapeutic target in colon, gastric, and triple-negative breast cancers [27, 55, 56]. CDH2 encodes cadherin-2 or N-cadherin as a transmembrane protein, and its overexpression can have a role in GR in NSCLC [33]. Overexpression of CDH2 on cancer cells facilitates their attachment to endothelial cells and leads to trans-endothelial migration of cancer cells [33]. SERPINE1 and CDH2 genes are associated with migration phenotype, a primary characteristic of drug-resistant tumors [2]. Clarifying the roles and mechanisms of SERPINE1 and CDH2 genes in regulating GR in LUAD will help to improve clinical treatment. In addition, the expression of SERPINE1 has significantly correlated with EGR1 and BMP2 (in PC9 cell) and JUN, CXCL12, and CAV1 (in HCC4006 cell). This correlation may indicate their involvement in common cellular pathways. Several studies suggested a possible role for the BMP2, JUN, CXCL12, and CAV1 genes in the metastatic evolution of lung cancer [16, 31, 39, 46]. As mentioned above, metastatic behavior has direct correlation with drug resistance in cancer. Further studies are necessary to investigate the functional interconnection of the mentioned genes and their relation with SERPINE1.

Because of the immunologic tolerance in the TME and the effect of immune infiltration on tumor development and drug resistance [57], we further explored whether hub genes correlate with immune cell infiltration. CAFs and immune cells are critical cellular components of the TME. Significant correlations between the expression of most of the hub genes and CAF tumor infiltrations in both cell lines were found. The high abundance of CAFs and macrophages in TME was reported to predict chemotherapy, radiotherapy, and immunotherapy resistance [58]. Therefore, by recruiting immune cells, the hub genes can indirectly lead to drug resistance, and targeting these genes for LUAD therapy can be proposed. For instance, targeting Pin1, an overexpressed gene in pancreatic cancer cells, inactivated CAFs and synergized with immunochemotherapy in the pancreatic cancer mice model [59]. Bilateral communication between cancer cells and other components of TME has been confirmed. It was shown that secreted cytokine by cancer cells can convert fibroblasts to inflammatory CAFs, by imposing modifications in TME, such as ECM components (remodeling architecture of TME) or providing survival signals to cancer cells to promote therapy resistance [60].

CONCLUSION

In conclusion, differential gene expression in drug-resistant cancer cells can induce alterations in tumor cell behavior and function and modify immune regulation and the ECM of the TME. For instance, the IL6R, CXCR4, CCL3, IRF7, MUC1, CD40, and EGR1 hub genes in PC9 cells are associated with cytokine signaling and might serve as novel therapeutic targets. The COL1A2, SERPINE1, COL5A1, LOX, COL6A1, and SPARC hub genes in HCC4006 cells are among the ECM-related genes, and their targeting may aid in the restoration of ECM abnormalities and the reversal of drug resistance. Additionally, the CXCR4, CD40, and LOX genes exhibited a higher frequency of positive correlation with CAFs and macrophage infiltrations in the TME. Immune infiltration can lead to modifications of both cellular and acellular components of the TME,

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ultimately contributing to drug resistance. Targeting the upregulated hub genes might effectively reverse the resistance of LUAD to gefitinib. Given the complex interconnections within the TME, simultaneous targeting of immune-related and ECM-related genes could potentially benefit LUAD patients in overcoming therapy resistance.

ACKNOWLEDGMENTS
The author thanks the University of Maragheh.

CONFLICT OF INTEREST
The author declares that no known competing financial interests or personal relationships could have appeared to influence the work reported in this paper.

ETHICS APPROVAL
Not applicable.

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