Identification of biomarkers, pathways and potential therapeutic targets for EGFR-TKI resistance in non-small-cell lung cancer

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Research Article

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Abstract

Background: Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) have been proven to provide survival benefits for non-small-cell lung cancer (NSCLC), but drug resistance represents a major therapeutic challenge. This study aimed to map the hub genes and potential pathways that might be involved in the molecular pathogenesis of EGFR-TKI resistance in NSCLC.

Methods: We performed a comprehensive bioinformatic analysis to identify differentially expressed genes (DEGs) between EGFR-TKI-sensitive and EGFR-TKI-resistant patient derived xenotransplantation (PDX) samples based on microarray data in the Gene Expression Omnibus (GEO) database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were applied for functional and pathway analysis of DEGs. The STRING database and Cytoscape software were used to construct protein–protein interaction (PPI) networks and determine gene interactions. Survival analysis was performed via the GEPIA database. The relationship between the EGFR-TKI resistance key gene ITGAM and the corresponding potential therapeutic candidates was retrieved from DGIdb.

Results: A total of 1302 DEGs were identified (P <.05, fold change >2) when comparing the EGFR-TKI resistant and sensitive groups based on the GSE64472 and GSE130160 datasets. GO functional analysis showed that these DEGs were mainly enriched in plasma membrane, integrin binding, cytokine activity, growth factor activity, platelet-derived growth factor binding immune response and cell adhesion. Pathway analyses also indicated that these DEGs are mainly involved in signaling by the chemokine-cytokine receptor interaction, melanogenesis and basal cell carcinoma. The PPI network highlighted 10 potential hub genes, including six upregulated genes, ITGAM, CCL5, CD4, IDO1, HAVCR2 and CCR7, and four downregulated genes, IL6, IL10, CXCL9 and TLR9. Only ITGAM was linked to poor DSF in NSCLC patients. A total of 10 drugs were predicted to be potential therapeutics for NSCLA with EGFR-TKI resistance.

Conclusion: The results of this study indicate that we have determined the hub genes related to EGFR-TKI resistance in NSCLC through bioinformatics technologies. Among them, ITGAM may play a role in the mechanism of resistance to EGFR-TKIs and helps to improve the understanding of the mechanisms of EGFR-TKI resistance and provide novel insights into therapeutics.

Introduction

Lung cancer is the second most commonly diagnosed cancer worldwide and the leading cause of cancer death (Sung et al., 2021). Non-small-cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers [1,2]. Targeted drugs represented by epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitors (TKIs) have brought revolutionary progress in the treatment of advanced NSCLC[3,4]. With the extensive clinical application of epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitors (TKIs), acquired resistance has become a challenge faced by clinicians[5,6]. Although numerous efforts

have been made to understand the molecular mechanisms of EGFR-TKI acquired drug resistance, the underlying molecular mechanisms and critical genes are still not completely clear[7,8].

With the rapid development of gene sequencing technology and bioinformatics analysis technology, researchers can access high-throughput microarray and next-generation sequence functional genomic data from the international public repository Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA)[9–11]. These online databases can obtain expression information of numerous genes simultaneously, and these genes were analyzed to explore potential biomarkers and therapeutic targets of EGFR-TKI drug resistance in non-small-cell lung cancer. However, the identification strategies of these markers largely depend on the comparison of normal and cancer tissue samples, especially the acquisition of drug-resistant samples, which are difficult to acquire. Recently, most sequencing studies have been limited to induced drug-resistant cell lines, which have limited value in analyzing the key genes involved in tumor drug resistance. The patient-derived xenotransplantation (PDX) model is a useful tool in cancer biology research based on its advantages in preserving the characteristics of patient tumors and is thus more suitable for use in experiments exploring the molecular mechanisms of tumor progression and drug resistance[12, 13]. To identify the genes responsible for EGFR-TKI drug resistance, we screened the gene chip data by using the GEO database. EGFR-TKI-sensitive and acquired resistant NSCLC public gene expression datasets (GSE64427 and GSE130160) were selected and downloaded from GEO.

In the present study, the differentially expressed genes (DEGs) between EGFR-TKI-sensitive and acquired drug-resistant non-small-cell lung cancer-transplanted tumor samples were obtained by mining the gene expression microarray datasets GSE64472 and GSE130160, and Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the DEGs were performed by using the Database for Annotation, Visualization and Integrated Discovery (DAVID) online tool. A protein–protein interaction (PPI) network of DEGs was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING) database and analyzed using Cytoscape software, and the hub genes were identified. In addition, the survival of patients with abnormal hub gene expression was analyzed using the TCGA database. The data will undoubtedly aid in the understanding of the roles of such genes in the development of EGFR-TKI resistance. The present study will contribute to the understanding of the molecular mechanism of EGFR-TKI resistance and provide new gene targets for future studies.

Materials And Methods

1. Microarray data

We screened the gene chip data by using the GEO (http://www.ncbi.nlm.nih.gov/geo) database, with the keywords “NSCLC” and “EGFR-TKI resistant” [14]. We screened PDX samples with EGFR mutations and sensitivity to EGFR-TKI drugs and induced acquired anti-EGFR-TKIs using a PDX model. Two datasets (GSE64472 and GSE130160) were selected for the analysis of differentially expressed genes (DEGs) between EGFR-TKI-sensitive and resistant samples in NSCLC. EGFR-TKI-resistant samples were characterized by a tumor volume tripled when compared to the pretreatment volume. GSE64472 included

3 EGFR-TKI-sensitive samples and 2 EGFR-TKI drug-resistant samples, and GSE130160 included EGFR-TKI-sensitive samples and 1 EGFR-TKI-resistant sample. The platforms used in these datasets were the GPL6884 Illumina HumanWG-6 v3.0 expression beadchip and the GPL16791 Illumina HiSeq 2500 (Homo sapiens) platform.

2. Screening of DEGs

The R (http://www.bioconductor.org/) “limma” package was used to normalize the data and perform differential expression analysis between EGFR-TKI-sensitive and acquired drug-resistant transplanted NSCLC tumor samples. DEGs were screened with a false discovery rate (FDR)-corrected p value < 0.05 and |log2-fold change (FC)| > 2. Before the analysis of DEGs between EGFR-TKI-sensitive and acquired drug-resistant transplanted tumor samples, the probe identification numbers were transformed into gene symbols. When multiple probes corresponded to the same gene, the max value was taken as the gene expression value.

3. Functional enrichment analysis of DEGs

To further clarify the potential functional roles and pathway enrichment associated with the DEGs, Gene Ontology (GO) analysis, including biological process, cellular component, and molecular function, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were completed with the Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/) (version 6.8) [12, 13][20]. Only terms with P values of < 0.05 and ≥ 2 enriched genes were considered statistically significant.

4. PPI Network Construction and hub gene identification

The PPI network of the DEGs was constructed using the online STRING (https://string-db.org) database to determine the molecular mechanisms of key signaling pathways and cellular activities in EGFR-TKI resistance in NSCLC[21].17 We uploaded all 1302 DEGs in the present study to yield an initial PPI and then visualized this network using Cytoscape Version 3.7.1. Relationships among DEGs were analyzed by the NetworkAnalyzer plug-in of Cytoscape software to characterize the small-world network by calculating the network properties, such as the clustering coefficient of the network, distribution of node degree and shortest path [23]. Next, the cytoHubb plug-in was used to rate the network, with the top 10 genes rated according to their degree, closeness, and betweenness scores being the candidate hub genes.

5. Hub Gene Survival Analysis

To identify the potential drug resistance role of these hub genes, the NSCLC patient disease-free survival (DFS) of hub genes was performed using the Kaplan–Meier curves in TCGA (https://portal.gdc.cancer.gov/) [16], and log rank test P value < 0.05 was the threshold of statistical significance. Using this approach, we were able to identify those genes associated with drug resistance progression in NSCLC.

6. Drug Interaction Prediction for EGFR-TKI Resistance-Specific Key Genes

Resistance-Specific Key Genes The Drug Gene Interaction Database (DGIdb; www.dgidb.org) was used to predict the interaction between genes and drugs. We uploaded the EGFR-TKI resistance-specific key genes into the DGIdb to obtain potential targeted drugs effective for EGFR-TKI resistance in NCLC.

Results

1. Identification of DEGs

To screen for meaningful biomarkers between the EGFR-TKI-sensitive and -resistant groups, using the R limma package and the $p < 0.05$ and $[logFC] > 2$ cutoff criteria, we detected a total of 1302 DEGs when comparing the EGFR-TKI-resistant and -sensitive groups based on the GSE64472 and GSE130160 datasets.773 DEGs were obtained in the resistant group of GSE64472, including 339 upregulated and 436 downregulated DEGs. A total of 529 DEGs were obtained, including 479 upregulated and 52 downregulated DEGs in the resistant group of GSE130160. The volcano plot of DEGs is presented in Fig. 1a, 1b, and the expression heatmap of the top 50 DEGs is presented in Fig. 1c, 1d (ranked by padj. value).

2. GO and KEGG enrichment analyses of DEGs

To clarify the role of these DEGs in the progression of EGFR-TKI resistance in NCLC, we immediately predicted the functional role of these genes. GO analysis, including cellular components (CC), molecular function (MF), biological process (BF), and KEGG analysis, was performed using the DAVID database to understand the functions of DEGs. With an FDR-corrected p value < 0.05 and enrichment score > 1.5 as the cutoff value, GO functional enrichment analysis resulted in a total of 1302 DEGs mapped to 48 significantly enriched functional clusters. In total, 11 GO terms were significantly enriched in cellular components, including 'plasma membrane', 'extracellular region', 'extracellular space', 'extracellular exosome' and 'proteinaceous extracellular matrix' (Fig. 2a). Enrichment of 8 GO terms, such as 'integrin binding', 'cytokine activity', 'growth factor activity', 'protein homodimerization activity', 'platelet-derived growth factor binding', 'calcium ion binding', 'extracellular matrix structural constituent' and 'transmembrane signaling receptor activity', belonged to molecular functions (Fig. 2b). A total of 29 biological processes were enriched, mainly involving 'immune response', 'cell adhesion', 'extracellular matrix organization', 'positive regulation of bone mineralization' and 'positive regulation of T-cell proliferation' (Fig. 2c). A total of 1303 DEGs were mapped into the KEGG database using DAVID, and enrichment score > 1.5 and p value < 0.05 were used as enrichment screening standards. In total, 16 enriched functional clusters of the DEGs were obtained (Fig. 2b), such as ‘cytokine–cytokine receptor interaction’ (34 genes), ‘melanogenesis’ (18 genes), ‘circadian entrainment’ (17 genes), ‘basal cell carcinoma’ (12 genes), and ‘dopaminergic synapse’ (20 genes).

3. Integration of the protein–protein interaction network and module analysis

The PPI network of 1302 DEGs was constructed and visualized using the STRING database. The isolated nodes and partially loosely connected gene nodes were removed, and the remaining DEGs together constituted a complex multicenter interaction network map, which contained 1402 nodes and 4761 edges (Fig. 3a). The average node degree was 7.92, and the average local clustering coefficient was 0.286. Among the 1402 nodes, the top 20 and top 10 DEGs with the highest degree of nodes were screened based on the Cytoscape software analysis results (Fig. 3b, 3c). The expression of the top 20 genes in GSE64472 and GSE130160 samples is shown in Fig. 3d and 3e. The results of the top 10 DEGs were as follows: IL6, IL10, CXCL9, ITGAM, CCL5, CD4, IDO1, HAVCR2, TLR9, and CCR7. The full name and function of these hub genes are listed in Table 1.

Table 1
Functional roles of 10 hub genes

No.	Gene symbol	Full name	Function
1	IL6	interleukin 6	a cytokine that functions in inflammation and the maturation of B cells.
2	IL10	Interleukin 10	a cytokine produced primarily by monocytes and to a lesser extent by lymphocytes. This cytokine has pleiotropic effects in immunoregulation and inflammation.
3	CXCL9	C-X-C Motif Chemokine Ligand 9	The protein encoded is thought to be involved in T-cell trafficking. The encoded protein binds to C-X-C motif chemokine 3 and is a chemoattractant for lymphocytes but not for neutrophils.
4	ITGAM	Integrin Subunit Alpha M	This gene encodes the integrin alpha M chain. This I-domain containing alpha integrin combines with the beta 2 chain (ITGB2) to form a leukocyte-specific integrin. The alpha M beta 2 integrin is important in the adherence of neutrophils and monocytes to stimulated endothelium, and in the phagocytosis of complement coated particles.
5	CCL5	C-C Motif Chemokine Ligand 5	This gene is one of several chemokine genes clustered on the q-arm of chromosome 17. This chemokine, a member of the CC subfamily, functions as a chemoattractant for blood monocytes, memory T helper cells and eosinophils.
6	CD4	CD4 molecule	the CD4 membrane glycoprotein acts as a coreceptor with the T-cell receptor on the T lymphocyte to recognize antigens displayed by an antigen presenting cell in the context of class II MHC molecules.
7	IDO1	Indoleamine 2,3-Dioxygenase 1	a heme enzyme that acts on multiple tryptophan substrates. This enzyme is thought to play a role in a variety of pathophysiological processes such as antimicrobial and antitumor defense, neuropathology, immunoregulation, and antioxidant activity.
8	HAVCR2	Hepatitis A Virus Cellular Receptor 2	The protein belongs to the immunoglobulin superfamily, and TIM family of proteins. CD4-positive T helper lymphocytes can be divided into types 1 (Th1) and 2 (Th2) on the basis of their cytokine secretion patterns.
9	TLR9	Toll Like Receptor 9	The protein encoded by this gene is a member of the Toll-like receptor (TLR) family, which plays a fundamental role in pathogen recognition and activation of innate immunity.
10	CCR7	C-C Motif Chemokine Receptor 7	The protein encoded by this gene is a member of the G protein-coupled receptor family. This receptor is expressed in various lymphoid tissues and activates B and T lymphocytes.

4. Disease-free surviva (DFS) analyses of hub genes in NSCLC

Based on the practicality of clinical guidance, we needed to find genes in these hub genes that could promote EGFR-TKI resistance and could be used for cancer progression prediction. We used the Kaplan–Meier Plotter database to explore how these hub genes were related to NSCLC patient DFS. Of these genes, we found that only elevated ITGAM expression was linked to better NSCLC patient DFS (HR = 0.73, 95% CI: 1.26–1.81, P = 0.045) (Fig. 4). Taken together, the results show that ITGAM functions as a core gene that has a close relationship with EGFR-TKI resistance.

5. Drug Interaction Prediction for EGFR-TKI resistance

The relationship between the EGFR-TKI resistance-specific gene ITGAM and the corresponding potential therapeutic candidates was retrieved from DGIdb. A total of 207 drugs were predicted to interact with ITGAM. Among them, the drugs with the highest number of target genes were liarozole, rovelizumab, dimethyl, sulfoxide, clarithromycin, fentanyl, phenylephrine, theophylline, morphine, hydrocortisone, and atorvastatin (Table 2).

Table 2
Top 10 drug predictions for the EGFR-TKI resistance-specific key gene TIMP1

Drug	Interaction Type & Directionality	Sources	Query Score	Interaction Score
Liarozole	n/a	NCI	2.92	4.25
Rovelizumab	antagonist (inhibitory)	ChemblInteractions	1.46	2.13
Dimethyl Sulfoxide	n/a	NCI	1.46	2.13
Clarithromycin	n/a	NCI	1.35	0.98
Fentanyl	n/a	NCI	0.67	0.49
Phenylephrine	n/a	NCI	0.63	0.91
Theophylline	n/a	NCI	0.37	0.53
Morphine	n/a	NCI	0.28	0.41
Hydrocortisone	n/a	NCI	0.23	0.34
Atorvastatin	n/a	NCI	0.15	0.21

Discussion

The molecular mechanism of EGFR-TKI resistance is largely divided into two categories: acquired resistance after EGFR-TKI treatment and primary resistance marked by cancer cell dependence on other

oncogenes, such as KRAS. The most common possible mechanism of acquired resistance is the development of an EGFR T790 M gatekeeper mutation, which occurs in 4 ~ 50% of cases [14–17]. Other reported mechanisms of acquired resistance include MET amplification [18], hepatocyte growth factor expression [19] and epithelial-mesenchymal transition [20]. Therefore, it is extremely important to identify the molecular mechanisms underlying the development of EGFR-TKI resistance in NSCLC and to provide novel gene targets for future treatment measures.

Cancer cell lines have been essential tools in drug screening for more than 25 years. However, cell lines have been cultured for thousands of generations and differ greatly from primary tumor tissues in genetic make-up and behavior[21]. Subcutaneous or orthotopic cell-derived tumor xenograft models (CDX models) cannot accurately mimic the tumor condition in human genetic heterogeneity. Medicine proved to be efficient in a traditional animal model [CDXmodels] and had a low response rate when used in clinical trials[22]. For the past several years, patient-derived xenograft models (PDX models) have attracted increased attention in preclinical cancer research and have emerged as promising tools for translational research. Investigators have shown that PDX models maintain most genetic features compared to primary human tumor tissue[12,13,23,24]. For pancreatic ductal adenocarcinoma (PDAC), the response to gemcitabine in PDX models showed a strong correlation with that in clinical patients [25]. In hepatocellular carcinoma (HCC), the efficacy of sorafenib in the HCC PDX model is similar to that in the primary patient [26, 27]. This suggests that PDXs are suitable models for the identification of molecular biomarkers related to drug sensitivity or resistance, as well as a screening tool for the efficacy of novel drugs.

Microarray technology is one of the most important approaches used by many researchers worldwide to explore the expression levels of genes involved in complex disorders[28]. The limitation of most previous bioinformatics studies is that they focused on the results from cell line-based or subcutaneous or orthotopic cell-derived tumor xenograft models. After a systematic search, two microarray datasets with an acquired resistance model in vivo were included in our study. In GSE64472 and GSE130160, EGFR-TKI-sensitive PDXs were initially sensitive to VEGFR inhibition but developed resistance following prolonged treatment with Vandetanib and osimertinib. We performed a series of bioinformatic analyses to compare the gene expression of EGFR-TKI-sensitive and EGFR-TKI-resistant PDX samples. The aim of our study was to identify and analyze the functions of hub genes in EGFR-TKI resistance, to help understand the molecular mechanisms underlying the development of drug resistance and to provide novel gene targets for the future.

In the present study, we used the intersection of the two study databases to obtain more reliable DEGs. Finally, 1203 DEGs were identified. GO functional analysis showed that these DEGs were mainly enriched in 'plasma membrane', 'extracellular region', 'integrin binding', 'cytokine activity', 'growth factor activity', 'protein homodimerization activity', 'platelet-derived growth factor binding', 'immune response', 'cell adhesion', and 'extracellular matrix organization'. Several studies have reported that integrin β3 is upregulated after EGFR-TKI treatment. Kanda and Wang found that acquired erlotinib resistance was mediated by the integrin β1/Src/Akt signaling pathway in lung cancer [29–31]. A recent study illustrated

that the FGFR inhibitor-resistant H1703 cell line is dependent on amplified platelet-derived growth factor receptor-α[32]. KEGG pathway analysis revealed that these DEGs are mainly involved in signaling by ‘cytokine–cytokine receptor interaction’, ‘melanogenesis’ and ‘basal cell carcinoma’. Interestingly, cytokine–cytokine receptor interactions in NSCLC have been shown to be one of the primary causes of EGFR-TKI resistance[33]. Loss of microphthalmia-associated transcription factor (MITF) is commonly observed in acquired resistance and is associated with a mesenchymal-like invasive phenotype or with a neural crest stem cell (NCSC) phenotype[34]. Studies have shown that the human prostate cancer cell line obtains enzalutamide resistance during treatment, and its phenotype changes from lumen epithelial cells to neuroendocrine and basal cells. This shows that the mechanism of differentiation into basal cells plays an important role in drug-induced resistance[35].

Using a PPI network, we identified 10 candidate hub genes (IL6, IL10, CXCL9, ITGAM, CCL5, CD4, IDO1, HAVCR2, TLR9, and CCR7) among these DEGs in our study. IL-10, an immunoregulatory component in the cytokine network, promotes tumor malignancy by promoting T-cell apoptosis and tumor cell survival [36]. Previous reports have shown that a persistently activated IL-6/STAT3 pathway promotes acquired resistance to targeted therapy with EGFR-TKIs in NSCLC treatment[37]. CXCL9, also known as MIG, is an inflammatory chemokine. A previous study also showed that the presence of CXCL9 in the tumor microenvironment inhibited NSCLC tumor growth and metastasis by decreasing tumor-derived angiogenesis, and high CXCL9 levels were found to be related to prolonged DFS and OS in early-stage lung adenocarcinoma patients [38]. In our study, the gene expression levels of CXCL9 were higher in EGFR-TKI-sensitive samples than in EGFR-TKI-resistant samples, which is in accordance with some results of previous studies showing that overexpression of CXCL9 could inhibit tumor-associated angiogenesis, playing a role in the progression of EGFR-TKI resistance. Human non-small-cell lung cancer is the third most frequent expresser of IDO1 after endometrial/cervical carcinomas and renal carcinomas. A recent study of an NSCLC cohort also showed that upregulation of IDO1 expression was significantly correlated with a higher pathologic stage as well as lymph node metastasis. These studies raise the possibility that IDO1 expression contributes to immune resistance and tumor progression[39]. CCL5 (chemokine ligand 5) is associated with the migration and metastasis of human cancer[40]. We used the online software GEPIA to perform survival analysis on 10 hub genes in the TCGA database. Only ITGAM was significantly associated with poor NSCLC patient prognosis, and high expression of ITGAM was associated with poor DFS. ITGAM is one of the genes associated with the development of the inflammatory response, also known as CD11b, Mac-1 integrin alpha chain or complement receptor 3. It is of key importance in regulating macrophage polarization and proinflammatory macrophage transcription, thereby restraining immunosuppressive macrophage polarization[41–43]. A recent study showed that ITGAM modulates angiogenesis through the control of cytokine expression in animal models of murine and human cancer[44]. The discovery of this result gave us the enlightenment that ITGAM regulates the occurrence of EGFR-TKI resistance directly or indirectly and may be used as a biomarker for the diagnosis of EGFR-TKI resistance.

Furthermore, we predicted the drugs regulating the EGFR-TKI resistance-specific gene ITGAM in NSCLC patients. Among the 10 predicted drugs for ITGAM, some have been reported to be effective in cancer therapy or combination therapy. Clarithromycin (CLM) inhibits autophagic flux and was reported to enhance the cytotoxic effect in NSCLC cell lines when combined with gefitinib (GEF)[45]. Another study suggested the possibility of using CLM as a 'chemosensitizer' for EGFR-TKI therapy in pancreatic cancer patients to enhance nonapoptotic tumor cell death induction[46]. Liarozole downregulates transforming growth factor (TGF)-alpha and EGFR levels in head and neck squamous cell carcinoma by increasing endogenous plasma concentrations of retinoid acid (RA)[47]. Several mechanisms of the antitumor activity of dimethyl sulfoxide (DMSO) have been reported [48]. For example, DMSO has antiangiogenic effects via inhibition of MMP-2 production and could mimic thalidomide to suppress choriocapillary endothelial cell proliferation[49]. Theophylline was reported to increase the sensitivity of H1299 lung cancer cells to the induction of cell death by gemcitabine and cisplatin[50]. Phenylephrine induced phosphorylation of EGFR, which was partially blocked by an EGFR tyrosine kinase inhibitor. Previous studies have shown that statins, HMG-CoA reductase inhibitors, enhance the tumor-inhibitory effect of many antitumor drugs; for example, a study suggested that atorvastatin sensitizes NSCLC cells to carboplatin through inhibition of AKT activation[51,52]. In addition, atorvastatin overcomes gefitinib resistance in KRAS mutant NSCLC cells through inhibition of HMG-CoA reductase-dependent disruption of the Kras/Raf and Kras/PI3K complexes[53]. Thus, the combination of atorvastatin and chemotherapy drugs in NSCLC treatment may reduce the resistance of patients. EGFR is coactivated by the μ-opioid receptor (MOR), which is expressed on NSCLC cells and human lung cancer. Preclinical studies have demonstrated that opioid receptor agonists increase the rate of non-small-cell lung cancer (NSCLC) growth and metastasis[54]. Therefore, it is extremely important to predict drugs that deserve further investigation in the treatment of EGFR-TKI resistance.

Conclusion

Our study analyzed gene expression between EGFR-TKI-sensitive and acquired drug-resistant samples from the GEO database and identified aberrant expression in EGFR-TKI-resistant PDXs. In this study, a total of 1302 DEGs and 10 hub genes were identified, and GO and KEGG enrichment analyses confirmed the functions and pathways of these DEGs. In addition, ITGAM might play important roles in the molecular pathogenesis of EGFR-TKI resistance. Furthermore, the core genes and pathways might be potential biomarkers that could be used for the detection and targeting of EGFR-TKI resistance for therapy. The predicted drugs have the potential to be used in combination with EGFR-TKIs to reduce resistance in NSCLC patients and improve therapeutic effectiveness. These findings may help in understanding the mechanisms of drug resistance and in discovering potential targets for EGFR-TKI resistance, which may help improve the therapeutic outcomes of NSCLC patients. However, further studies are still needed to conduct a series of experimental studies to prove this hypothesis to obtain more precise correlation reports.

Abbreviations

EGFR-TKIs: Epidermal growth factor receptor-tyrosine kinase inhibitors; NSCLC: non-small-cell lung cancer; DEGs: Diferentially expressed genes; FC: Fold change; FDR: False discovery rate; GO: Gene Ontology; CC: Cellular components; MF: molecular function; BF: Biological process; KEGG: Kyoto Encyclopedia of Genes and Genomes; PDX: Patient derived xenotransplantation; TCGA: The Cancer Genome Atlas; DAVID: Database for Annotation, Visualization and Integrated Discovery; DFS: Disease-free survival.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Ying Wang conceived and designed the study and edited the final draft. Leilei Zhu and Shanshan Gao carried out the literature review and prepared the draft manuscript; Leilei Zhu and Xianya Zhao helped in preparing the manuscript and its editing. The authors read and approved the final manuscript.

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Figures

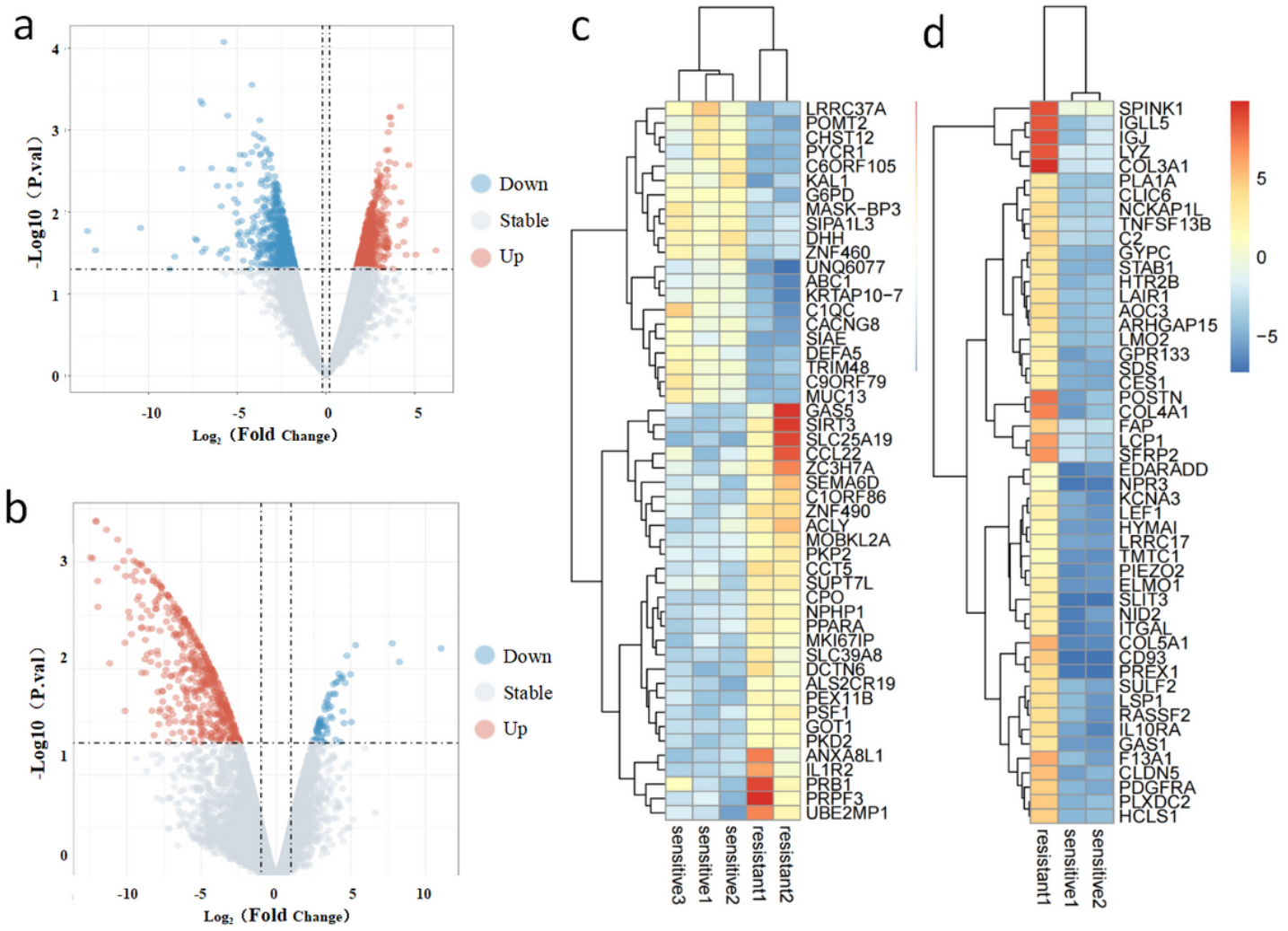

Figure 1

Identification of differentially expressed genes. (a, b) Volcano plot of DEGs in the GSE64472 and GSE130160 datasets. The red dots represent upregulated genes, the green dots represent downregulated genes, and the black dots represent genes with no significant difference in expression. (c, d) Heatmap of the top 50 DEGs in the GSE64472 and GSE130160 datasets. Red represents upregulated genes, and blue represents downregulated genes.

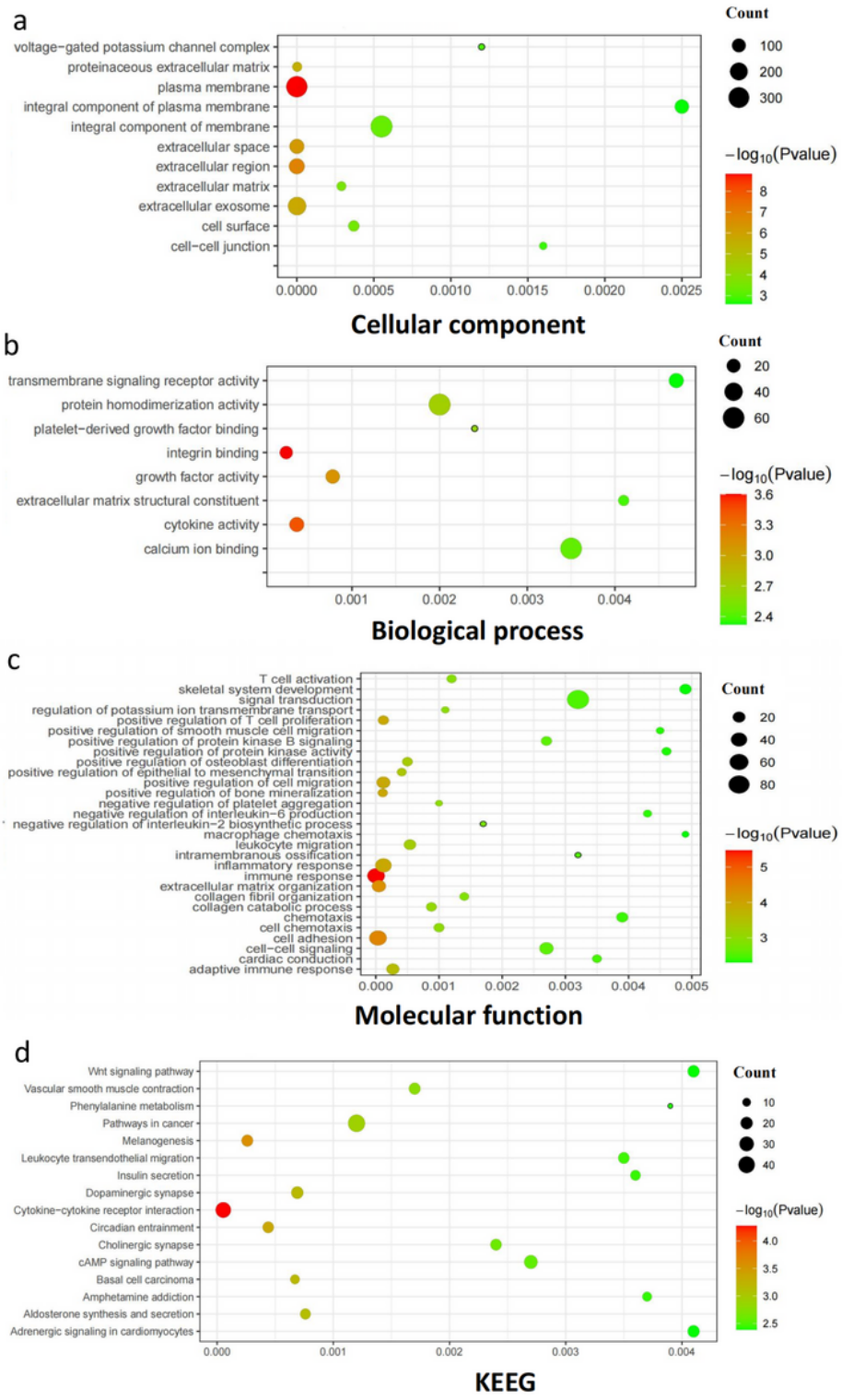

Figure 2

Gene Ontology and KEGG pathway analysis of DEGs in NSCLC. (a) GO covering the domains of molecular functions (MF). (b) GO covering the domains of biological processes (BP). (c) GO covering the domains of cellular components (CC). (d) KEGG pathways that were the most significantly upregulated pathways during SCLC. The bubbles represent the enrichment pathway with p values <0.05. The bubble size represents the number of enriched target genes in the process. The bubble coulor represents −log10

(p value); from green to red, the p value decreases. The Y-axis represents the enrichment target of GO or pathway. The X-axis is the Richfactor: it is counts divided by the third column.

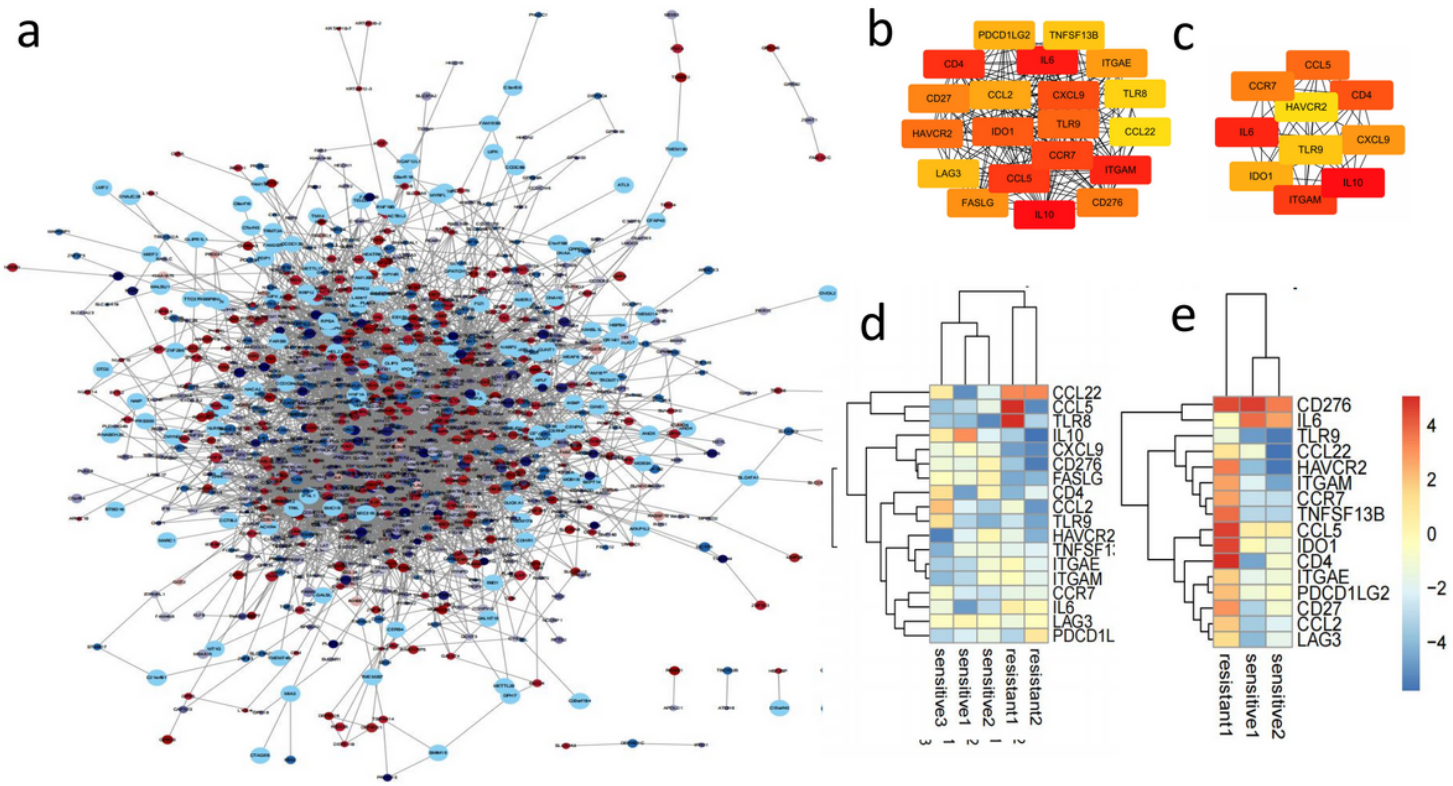

Figure 3

PPI analysis of DEGs based on Cytoscape. (a) Visualized PPI analysis of DEGs. (b) Top 20 genes with the highest MCC scores in DEGs. (c) Top 10 genes with the highest MCC scores in DEGs; a darker color represents higher MCC scores. (d) Heatmap of the top 20 DEGs in GSE64472. (e) Heatmap of the top 20 DEGs in GSE130160. Red represents upregulated genes, and blue represents downregulated genes.

Figure 4

Disease-free survival analyses of 10 hub genes based on The Cancer Genome Atlas