Research Article

Identification of Significant Genes in Lung Cancer of Nonsmoking Women via Bioinformatics Analysis

Yu Wang,1 Sibo Hu,2 Xianguang Bai,1 Ke Zhang,1 Ruixue Yu,1 Xichao Xia,1 and Xinhua Zheng1

1College of Medicine, Pingdingshan University, Pingdingshan, Henan, China
2Department of Physical Education, Henan University of Urban Construction, Pingdingshan, Henan, China

Correspondence should be addressed to Xinhua Zheng; wawdym819@126.com

Received 3 March 2021; Accepted 26 August 2021; Published 11 October 2021

Academic Editor: Kui Li

Copyright © 2021 Yu Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. The aim of this study was to identify potential key genes, proteins, and associated interaction networks for the development of lung cancer in nonsmoking women through a bioinformatics approach. Methods. We used the GSE19804 dataset, which includes 60 lung cancer and corresponding paracancerous tissue samples from nonsmoking women, to perform the work. The GSE19804 microarray was downloaded from the GEO database and differentially expressed genes were identified using the limma package analysis in R software, with the screening criteria of p value < 0.01 and |log2 fold change (FC)| > 2. Results. A total of 169 DEGs including 130 upregulated genes and 39 downregulated were selected. Gene Ontology and KEGG pathway analysis were performed using the DAVID website, and protein-protein interaction (PPI) networks were constructed and the hub gene module was screened through STING and Cytoscape. Conclusions. We obtained five key genes such as GREM1, MMP11, SPP1, FOSB, and IL33 which were strongly associated with lung cancer in nonsmoking women, which improved understanding and could serve as new therapeutic targets, but their functionality needs further experimental verification.

1. Introduction

Until recently, lung cancer (LC) is the malignant neoplasm with the highest incidence and mortality worldwide, the tumor with the highest cancer mortality rate among men and the second highest cancer mortality rate among women [1]. Smoking is the major independent risk factor in the development of LC [2, 3]. Nonetheless, 15% of men and 53% of women with LC have never consumed tobacco [4]. Among them, nonsmokers are more common in women with lung cancer [5]. Therefore, there might be many other important factors that affect the occurrence and development of lung cancer in nonsmokers, such as air pollution, second-hand smoke, genetic factors, and occupational exposure. Although many genes have been screened to understand the causes of lung cancer in nonsmoking female patients, such as TP53 [6], PI3K [7], EML4-ALK [8], and BIRC5 [9], the molecular mechanism is still unclear. It is crucial to recognize the unique molecular phenotypic characteristics of nonsmokers with lung cancer for early diagnosis and targeted therapy.

With the development of gene microarray technology and the application of bioinformatics tools, whole gene expression profiling can be used to compare the expression changes of thousands of genes simultaneously and comprehensively screen all relevant genes of cancer, as well as to reveal the interrelationship between different gene expression changes, thus providing clues for studying the intrinsic connection between genes [10–12]. Bioinformatics-based data analysis plays an important role in the study of oncology [13]. In recent years, a large number of gene microarray datasets have been developed from lung cancer specimens, from which a series of differentially expressed genes (DEGs) have been identified, and gene annotation and pathway
functions have been carried out [14, 15]. The analysis of DEGs may provide a possibility for diagnosis marker and therapeutic targets at the molecular level of LC.

In this work, we acquired mRNA expression profiles from the GSE19804 dataset through the GEO website (https://www.ncbi.nlm.nih.gov/geo/), which is a public database that allows archiving, uploading, and querying microarrays. A total of 60 nonsmoking women with lung cancer were absorbed in GSE19804 [16, 17], and samples were collected from tumor (marked cancer) and adjacent normal tissue (marked normal). We used the limma [18] package built in R [19] software to obtain DEGs from mRNA expression profiling data and categorized them into up- and downregulated genes. Then, gene function and pathway analysis of DEGs was performed with DAVID (https://david.ncifcrf.gov/). Protein-protein interaction (PPI) network was conducted by STRING [20] (https://string-db.org/) and visualized by Cytoscape [21]. The core gene module (Module 1) of the network was identified by MCODE [22] app. Ultimately, we performed overall survival analysis for each gene in Module 1 via the Kaplan-Meier Plotter [23] (https://kmplot.com/analysis/). By applying this approach, the genes that were flitted may be associated with the development of lung cancer in nonsmoking women, which were identified as potential biomarkers for diagnosis, prognosis, therapeutic targets, and clinical pharmaceutical research. The framework of this study is shown in Figure 1.

2. Methods and Materials

2.1. Data Acquisition and Preprocessing. The microarray data GSE19804 [16, 17] was downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The platform of GSE19804 was Affymetrix Human Genome U133 Plus 2.0 Array, which contained 60 female lung cancer patients who have never smoked and 60 normal controls. mRNA expression matrices of patients can be obtained from the microarray. All the samples were collected from Taiwan. Subsequently, the probe identification numbers were transformed into official gene symbols. After deletion of duplicate genes, one-to-many and non-mRNA probes, the next differential gene analysis was performed on all gene expression data.

2.2. Identification of DEGs. DEG analysis is the finding of statistically significant genes from the multitude of genetic information on gene expression microarrays. All the gene expression data were analyzed using the limma package of R Studio [19, 24] software. The limma [18] package performs differential analysis of gene expression data and
experimental design through linear modeling. We applied the limma package for preliminary DEG screening of tumor tissues (marked cancer) and adjacent normal tissues (marked normal). With the parameters of the filter set to $|\log_2 \text{fold change}| \geq 2$ and adjust $p$ value $< 0.01$, the resulting DEGs would proceed to the next step.

2.3. Functional and Signal Pathway Enrichment Analysis. Gene Ontology (GO) [25] is the standardized portrayal or semantic interpretation of terms used to characterize genes and their products, including biological process (BP), cellular component (CC), and MF (molecular function). The KEGG pathway [26] is a set of manually drawn pathway maps representing our understanding of molecular interactions, reactions, and networks of relationships in metabolism, genetic information processing, etc. We used the online tool DAVID [27] website (https://david.ncifcrf.gov/) for GO and KEGG pathway annotation of candidate genes, setting the terms to "Homo sapiens," $p$ value $< 0.05$.

2.4. Protein-Protein Interaction Analysis and Gene Module Analysis. To understand the interactions between DEGs,
PPI queries were conducted. PPI analysis was carried out using STRING [20, 28] (https://string-db.org/, version 11.0) database, which is an online tool to search known protein interactions. We uploaded DEGs to the system and set the minimum required interaction score > 0.150 as restriction. The active interaction sources included text mining, experiments, databases, coexpression, neighborhood, gene fusion, and cooccurrence. The results from the PPI analysis were visualized by applying the Cytoscape [21] software (version 3.7.2). Subsequently, an app named MCODE [22] (https://apps.cytoscape.org/apps/mcode, version 1.6.1) was utilized to find the core module in the networks. The MCODE parameters were set to the default value, except \(K\)-core = 6.

### 2.5. Overall Survival Analysis Validation and Gene Ontology
To verify whether hub gene expression has survival

| Category                        | Term | Count | \(p\) value | Gene name |
|---------------------------------|------|-------|-------------|-----------|
| GOTERM_BP_DIRECT                | GO:0030574–collagen catabolic process | 7    | 2.08E–05    | COL6A6, COL1A1, COL11A1, MMP12, MMP1, COL10A1, MMP1 |
| GOTERM_BP_DIRECT                | GO:0001937–negative regulation of endothelial cell proliferation | 5    | 1.16E–04    | XDH, CAV2, CAV1, RGCC, SULF1 |
| GOTERM_BP_DIRECT                | GO:0003198–extracellular matrix organization | 9    | 3.49E–04    | RXFP1, SPOCK2, FOXF1, COMP, COL1A1, COL11A1, ABI3BP, COL10A1, SPP1 |
| GOTERM_BP_DIRECT                | GO:0050729–positive regulation of inflammatory response | 6    | 4.61E–04    | AGTR1, S100A8, IL1RL1, FABP4, IL33, S100A12 |
| GOTERM_BP_DIRECT                | GO:0031623–receptor internalization | 5    | 5.47E–04    | RAMP3, CAV1, CD36, CXC2R, CALCRL |
| GOTERM_CC_DIRECT                | GO:0005576–extracellular region | 41   | 1.02E–09    | TCF21, AGTR1, SIX1, SULF1, MME, ADAMTS1, EMCN, S100A8, CXCL2, IL33, MMRN1, MMP1, IGSF10, OGN, COL6A6, FCN3, BCHE, COMP, TEK, SFTPD, SFTPC, FAM150B, CFD, FIBIN, COL11A1, HBB, THBS2, SPP1, COL10A1, IL6, KL, AGER, MMP12, PLAC9, S100A12, MMP11, PCOLCE2, CHRD1L, CXCL14, PPBP, CXCL13, TGFBR3, HBEFG, WIFI, COL1A1, CP, MFP4 |
| GOTERM_CC_DIRECT                | GO:0005578–proteinaceous extracellular matrix | 17   | 1.87E–09    | CTHRC1, MAMDC2, ADAMTS3, SPOCK2, IL1RL1, MMP1, MMP12, MMP1, OGN, COL6A6, COMP, CCB1, SFTPD, TGFBR3, ADAMTS1, COL11A1, COL10A1 |
| GOTERM_CC_DIRECT                | GO:0005615–extracellular space | 36   | 4.65E–09    | XDH, CTHRC1, S100A8, CXCL2, SPINK1, IL33, GREM1, SCGB1A1, ABI3BP, OGN, COMP, SOSTDC1, CCB1, SFTPD, SFTPC, CFD, FGFBP2, GOLM1, SPP1, IL6, KL, GNLY, CTS1, C2ORF40, GKN2, CD36, CXCL14, PPBP, CXCL13, SULF1, TGFBR3, HBEFG, COL1A1, CP, LRK2, CPB2 |
| GOTERM_CC_DIRECT                | GO:0005581–collagen trimer | 10   | 1.15E–07    | CTHRC1, CD36, COL6A6, FCN3, CCB1, SFTPD, COL1A1, COL11A1, MMP1, COL10A1 |
| GOTERM_CC_DIRECT                | GO:0006323–basolateral plasma membrane | 9    | 1.93E–04    | CAV1, PROM2, TEK, CD300LG, AQP4, CA4, CEACAM5, GPHBP1, LIN7A |
| GOTERM_CC_DIRECT                | GO:0045121–membrane raft | 9    | 4.79E–04    | EDNRB, CAV2, CAV1, PROM2, CD36, SDPR, SLCE6A4, SULF1, TEK |
| GOTERM_MF_DIRECT                | GO:0008201–heparin binding | 10   | 7.06E–06    | OGN, CXCL13, COMP, TGFBR3, HBEFG, ADAMTS1, THBS2, AGER, ABI3BP, PCOLCE2 |
| GOTERM_MF_DIRECT                | GO:0008099–chemokine activity | 4    | 0.007634616 | PPBP, CXCL14, CXCL13, CXCL2 |
| GOTERM_MF_DIRECT                | GO:0005518–collagen binding | 4    | 0.01328243  | COMP, CCB1, ABI3BP, PCOLCE2 |
| GOTERM_MF_DIRECT                | GO:0004222–metalloendopeptidase activity | 5    | 0.01426339  | MME, ADAMTS1, MMP12, MMP1, MMP1 |
| GOTERM_MF_DIRECT                | GO:0017134–fibroblast growth factor binding | 3    | 0.015208209 | CXCL13, KL, TGFBR3 |
| GOTERM_MF_DIRECT                | GO:0005509–calcium ion binding | 13   | 0.015389499 | S100A8, TNNC1, SPOCK2, COMP, SULF1, CCB1, TNNC1, THBS2, MMP1, CDH5, MMP12, S100A12, MMP1 |

Table 2: Gene Ontology of DEGs associated with nonsmoking woman lung cancer.
Figure 3: The Gene Ontology and KEGG pathway of DEGs. (a) The top 6 functional enrichment analysis of DEGs in biological process (BP), cellular component (CC), and molecular function (MF). (b) The top 6 functional enrichment analysis arranged according to adj. p value.
significance in nonsmoking female lung cancer patients, overall survival analysis was performed. The genes in the previous module were verified for overall survival analysis with the Kaplan-Meier Plotter [23] (https://kmplot.com/analysis/). This online tool integrated the gene expression and clinical data from GEO, EGA, and TCGA, including lung cancer \((n = 3452)\), which can evaluate the impact of 54k genes (mRNA, miRNA, and protein) on cancer survival. The Kaplan-Meier survival chart was conducted to compare the two patient cohorts, and the 95% confidence interval and logrank value hazard ratio were calculated. We selected the types of diseases as “lung cancer,” setting limits as follows: “gender: female,” “smoking history: only those never smoked,” “split patients by the following: lower tertile” and “logrank value < 0.05.” Survival was evaluated using the Kaplan-Meier survival curves. Ultimately, the genes verified by survival analysis were subjected to GO analysis using Metascape [29] (https://metascape.org/gp/index.html).

3. Results

3.1. Identification of DEGs. By analyzing the gene expression microarrays GSE19804, with criteria as \(|\log_2 FC| \geq 2\) and adjust \(p\) value < 0.01, a total of 169 DEGs were selected, including 39 upregulated genes and 130 downregulated genes (Figure 2 and Table 1). The top 10 upregulated genes are:

| Term | Count | \(p\) value | Gene name |
|------|-------|-------------|-----------|
| hsa05144: malaria | 6 | 1.27E-04 | IL6, CD36, COMP, ACKR1, THBS2, HBB |
| hsa04512: ECM-receptor interaction | 7 | 2.36E-04 | CD36, COL6A6, COMP, COL1A1, THBS2, SPP1 |
| hsa04974: protein digestion and absorption | 6 | 0.001924368 | COL6A6, MME, COL1A1, CPB2, COL11A1, COL10A1 |
| hsa04510: focal adhesion | 8 | 0.004729076 | CAV2, CAV1, COL6A6, COMP, COL1A1, THBS2, SPP1 |
| hsa04151: PI3K-Akt signaling pathway | 9 | 0.023099212 | IL6, COL6A6, COMP, TEK, GNG11, COL1A1, THBS2, SPP1 |
| hsa03232: PPAR signaling pathway | 4 | 0.030418433 | CD36, FABP4, ACADL, MMP1 |
| hsa04062: chemokine signaling pathway | 6 | 0.04032297 | PPBP, CXCL14, CXCL13, CXCL2, CXCR2, GNG11 |

Table 3: Significantly enriched KEGG pathway of differentially expressed genes (DEGs).
Figure 5: The protein-to-protein interaction network. (a) The overview of the PPI network, with 167 nodes/genes and 1357 edges, including 129 downregulated (marked blue) and 38 upregulated (marked red) genes. The color shade of the nodes was set according to log FC p value of DEGs, and the size was set according to the edges. (b) Module 1 consisted of 16 nodes/genes.
were SPP1, COL11A1, COL10A1, HS6ST2, SPINK1, TOX3, CTHRC1, MMP12, MMP1, and GREM1. The top 10 downregulated genes were MMRN1, KCNK3, RXFP1, RAMP3, SOCS2, FOXF1, FIBIN, KANK3, HBEGF, and PCOLCE2.

3.2. Functional and Signal Pathway Enrichment Analysis. By performing GO and KEGG pathway analysis of DEGs through the DAVID website, we classified them into three terms: biological process (BP), cellular component (CC), and molecular function (MF). It can be seen from Table 2 and Figure 3. The top 3 significant distributions of BP enrichment were “collagen catabolism,” “negative regulation of endothelial cell proliferation,” and “extracellular matrix tissue”; CC were “extracellular area,” “protein extracellular matrix,” and “extracellular space”; and MF were “heparin binding,” “chemokine activity,” and “collagen binding,” respectively. Through the analysis of the KEGG pathway on the DAVID website, the DEGs were enriched in the following 7 pathways (Figure 4 and Table 3). The top 3 pathways with the largest differences were “malaria,” “ECM-receptor interaction,” and “protein digestion and absorption.”

3.3. Protein-Protein Interaction Analysis and Gene Module Analysis. We uploaded 169 DEGs to the STRING website and deleted the disconnected nodes to form a network of 167 nodes/genes and 1357 edges, including 129 downregulated and 38 upregulated genes. Then, the network was imported into the Cytoscape software for visualization (Figure 5(a)). Moreover, based on MCODE, with K-core = 6 as criteria, the most significant gene module (Module 1) was selected, which contained 16 node/genes and 58 edges (Figure 5(b)). In Module 1, 9 genes were upregulated respectively. Through the analysis of the KEGG pathway on the DAVID website, the DEGs were enriched in the following 7 pathways (Figure 4 and Table 3). The top 3 pathways with the largest differences were “malaria,” “ECM-receptor interaction,” and “protein digestion and absorption.”

Figure 6: The overall survival of the 5 genes in female LC of nonsmokers. Downregulated genes ((a) FOSB, (b) IL33) and upregulated genes ((c) GREM1, (d) MMP11, and (e) SPP1).
women tissue without tobacco consumption with the adja-
explore novel potential genes through comparing 60 LC
patients is not clear. The purposes of our study were to
the mechanism related to nonsmoking female lung cancer
could be classi
dent risk factor for LC, but a proportion of lung cancer
methods for exploring gene expression and is particularly
Gene microarray technology is one of the most important
Gene Ontology of 5 hub genes through Metascape.

| Gene   | Gene full name   | Biological process (GO)                                               | GO term          |
|--------|------------------|-----------------------------------------------------------------------|------------------|
| FOSB   | Proto-oncogene   | Response to corticosterone                                            | GO:0051412       |
|        |                  | Response to isoquinoline alkaloid                                      | GO:0014072       |
|        |                  | Response to morphine                                                  | GO:0043278       |
|        |                  | Regulation of bone trabecula formation                               | GO:1900154       |
| IL33   | Interleukin 33   | Negative regulation of bone trabecula formation                       | GO:1900155       |
|        |                  | Negative regulation of osteoclast proliferation                       | GO:0090291       |
| GREM1  | Gremlin 1        | Regulation of cellular defense response                               | GO:0010185       |
|        |                  | Positive regulation of cellular defense response                      | GO:0010186       |
|        |                  | Negative regulation of macrophage proliferation                       | GO:0120042       |
|        |                  | Basement membrane organization                                        | GO:0071711       |
| MMP11  | Matrix metallopeptidase 11 | Collagen catabolic process                                            | GO:0030574       |
|        |                  | Negative regulation of fat cell differentiation                       | GO:0045599       |
|        |                  | Collateral sprouting of intact axon in response to injury             | GO:0048673       |
| SPP1   | Secreted phosphoprotein 1 | Regulation of collateral sprouting of intact axon in response to injury| GO:0048683       |

(COL10A1, COL11A1, COL1A1, CXCL13, CXCL14, GREM1, MMP11, SPP1, and THBS2) and 7 genes were downregulated (FOSB, IL33, LYVE1, PPBP, S100A12, and S100A8). The 16 genes above would be selected for the next step verification.

3.4. Overall Survival Analysis Validation and Gene Ontology. In order to evaluate the clinical significance of these genes in nonsmoking female lung cancer patients, we imported the 16 candidate genes into the Kaplan-Meier Plotter for overall survival analysis verification. Using the selected parameters, the analysis runs on 168 patients. As shown in Figure 6, there were 5 genes that meet the screening requirements. The upregulated genes of GREM1 (HR = 3.82) (Figure 6(c)), SPP1 (HR = 3.6) (Figure 6(d)), and MMP11 (HR = 4.93) (Figure 6(e)) had lower survival rate in the high expression group compared with the low expression group, while the downregulated genes of FOSB (HR = 0.45) (Figure 6(a)) and IL33 (HR = 0.35) (Figure 6(b)) were the opposite. Gene Ontology of 5 hub genes was performed through Metascape (Table 4).

4. Discussion
Gene microarray technology is one of the most important methods for exploring gene expression and is particularly relevant in the study of complex refractory diseases [30]. It is well known that smoking is the most important independent risk factor for LC, but a proportion of lung cancer patients have never smoked, which is more frequent among females. As previously described, nonsmoking lung cancer could be classified as a unique type according to the unique genome and molecular mechanism [31]. Although many genes, such as CYP1A1 [32], ERCC2 [33], and L10 [34], have been confirmed to relate to nonsmoking lung cancer, the mechanism related to nonsmoking female lung cancer patients is not clear. The purposes of our study were to explore novel potential genes through comparing 60 LC women tissue without tobacco consumption with the adja-
cent normal tissue. By using R software, we identified 39 upregulated and 130 downregulated DEGs from GSE19804 downloaded from the GEO database. Following GO and KEGG pathway analysis, PPI network of DEGs was performed and the most significant gene module (Module 1) was selected, from which 16 genes were chosen to validate overall survival in the Kaplan-Meier Plotter. Finally, a total of 5 genes, GREM1, MMP11, SPP1, FOSB, and IL33, were screened out as potential biological markers.

We implemented GO and KEGG pathway analysis using the DAVID online tool to identify BP, CC, and MF and pathways involved in DEGs. With regard to BP, DEGs are mainly enriched in collagen catabolic process, extracellular matrix organization, and positive regulation of inflammatory response. In fact, collagen metabolism and extracellular matrix organization are widely involved in the growth, metastasis [35], and immunosuppression [36] of lung cancer. The genes identified in this study have been shown to be associated with collagen metabolism in tumors, with mmp11 leading to LC progression through regulation of collagen catabolism and fibrous tissue. DEGs in CC are majorly enriched in extracellular region, proteinaceous extracellular matrix, and collagen trimer. In consonance with this, DEGs are predominantly associated with collagen binding and chemokine and metalloendopeptidase activity in MF. Collagen provides a scaffold for extracellular matrix (ECM) assembly and promotes cancer cell migration and invasion [37]. It has also been reported that intratumoral collagen is a major source of immunosuppression and resistance to PD-1/PD-L1 axis blockade [37]. The genes identified in this study have been shown to be involved in the promotion of collagen metabolism in LC, with MMP11 leading to LC progression through regulation of collagen catabolism and fibrous tissue [38].

Next, interrelationship analysis of the pathway was performed using the KEGG process in DAVID. DEGs were mainly associated with ECM-receptor interaction including COL11A1, COL1A1, THBS2, and SPP1. The ECM can be classified into interstitial matrix (IM) and basement
membrane (BM), in which renewal and degradation are intrinsically linked to the invasive phenotype of malignant cells [39]. Furthermore, COL11A1, encoding collagen type XI α1, was overexpressed in recurrent and metastatic NSCLC and promotes proliferation, invasion, and migration of NSCLC via the Smad signaling pathway [40]. Additionally, SPP1 (osteopontin) was enriched in both the ECM-receptor interaction and PI3K-Akt signaling pathway. SPP1 is an important component of ECM, regulating matrix interactions and cell adhesion [41]. SPP1 promotes tumorogenesis and metastasis through accumulation of vascular endothelial growth factor (VEGF) [42] and facilitates immune escape from tumors through upregulation of PD-L1 tumor-associated macrophages [43]. Also, fibroblasts differentiated from bone marrow CD4+ monocytes enhance the cancer hepatocyte-like properties of LC cells through the secretion of SPP1 and activation of the PI3K/AKT pathway [44]. We also found that PI3K/AKT was a significantly enriched pathway. Activation of the PI3K/AKT pathway may lead to upregulation of tumors via VEGF, resulting in tumors with angiogenic properties [45]. In consensus, both SPP1 and COL11A1 were found as upregulated DEGs in this work, indicating that the ECM-receptor interaction and PI3K/AKT signaling pathway might play a key role in nonsmoking female patients of LC.

Although the GSE19804 datasets have been mined several times, our work focused on gene expression differences in lung cancer in nonsmoking women and therefore has certain uniqueness in terms of data mining perspectives, methods, and results compared to existing studies. Firstly, the perspective of analysis is different, as the main way of using these datasets was to study the gene expression differences between non-small-cell lung cancer (NSCLC) and normal tissues, without using smoking as a qualifying study condition [46–49]. Secondly, many studies target the significance of single gene expression in NSCLC, especially oncogenes, such as cyclin B2 (CCNB2) [50], pituitary tumor transforming gene-1 (PITG1) [51], and tumor suppressor gene as hedgehog-interacting protein (HHIP) [52]. Of course, there are also studies on nonsmoking lung cancer in women. In screening for DEGs, we used the limma package in R software, but Yang et al. [9] used the GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), which is an online tool of the GEO database. So we screened for different DEGs and pathways. In addition, some studies are screening for miRNA [53] and lncRNA [54].

5. Conclusion
This study investigated the potential candidate genes and signaling pathways of DEGs in lung cancer with nonsmoking women by analyzing the GSE19804 microarrays. Genes were selected by DEG, GO, KEGG, and PPI analysis. Finally, the upregulated (GREM1, MMP11, and SPP1) and downregulated (FOSB, IL33) genes were screened. This study improves our understanding of the pathogenesis and underlying molecular mechanisms of lung cancer in nonsmoking women. These selected candidate genes and pathways could give us a clue for a new therapeutic target. However, determining the function of these molecules requires further molecular biology experimental validation.

Abbreviations
- BP: Biological process
- CC: Cellular component
- DEGs: Differentially expressed genes
- ECM: Extracellular matrix
- GEO: Gene Expression Omnibus
- GO: Gene Ontology
- GREM1: Gremlin 1
- KEGG: Kyoto Encyclopedia of Genes and Genomes
- LC: Lung cancer
- MMP11: Matrix metallopeptidase 11
- IL33: Interleukin 33
- PPI: Protein-protein interaction
- FOSB: Proto-oncogene
- SPP1: Secreted phosphoprotein 1
- VEGF: Vascular endothelial growth factor.

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare that there is no conflict of interests.

Authors’ Contributions
All authors participated in this work. Sibo Hu and Yu Wang performed bioinformatics analysis on the data. Yu Wang integrated the data and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgments
We thank the GEO database for providing the raw microarray data for this study. Thanks are due to Bin Zhao of Sheng Xin Zhu Shou (official WeChat ID: SCIPhD) for his suggestions on the article. The research, writing, and/or publication of this paper were supported by the following funds. This work was supported by the Henan Provincial Science and Technology Tackling Project (No. 192102310087).

References
[1] L. A. Torre, R. L. Siegel, and A. Jemal, “Lung cancer statistics,” in Lung Cancer and Personalized Medicine, vol. 893 of Advances in Experimental Medicine and Biology, , pp. 1–19, Springer, 2016.
[2] J. Jeon, T. R. Holford, D. T. Levy et al., “Smoking and lung cancer mortality in the United States from 2015 to 2065,” Annals of Internal Medicine, vol. 169, no. 10, pp. 684–693, 2018.
[3] R. M. Hoffman and R. Sanchez, “Lung cancer screening,” Medical Clinics of North America, vol. 101, no. 4, pp. 769–785, 2017.
[4] L. Corrales, R. Rosell, A. F. Cardona, C. Martín, Z. L. Zatarain-Barrón, and O. Arrieta, "Lung cancer in never smokers: the role of different risk factors other than tobacco smoking," Critical Reviews in Oncology/Hematology, vol. 148, article 102895, 2020.

[5] G. A. Rivera and H. Wakelee, "Lung cancer in never smokers," in Lung Cancer and Personalized Medicine, vol. 893 of Advances in Experimental Medicine and Biology, , pp. 43–57, Springer, 2016.

[6] S. Toyooka, T. Tsuda, and A. F. Gazdar, "TheTP53 gene, tobacco exposure, and lung cancer," Human Mutation, vol. 21, no. 3, pp. 229–239, 2003.

[7] J. Kirby, P. R. Heath, P. J. Shaw, and F. C. Hamdy, "bioinformatics analyses, nonsmoking females with non-small cell lung carcinoma by Mutations and copy number gains in human lung cancers," vol. 385, 2016.

[8] D. W. Wong, E. L. Leung, K. K. So et al., "TheEML4–ALKfusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-typeEGFRandKRAS," Cancer, vol. 115, no. 8, pp. 1723–1733, 2009.

[9] G. Yang, Q. Chen, J. Xiao, H. Zhang, Z. Wang, and X. Lin, "Identification of genes and analysis of prognostic values in nonsmoking females with non-small cell lung carcinoma by bioinformatics analyses," Cancer Management and Research, vol. 10, pp. 4287–4295, 2018.

[10] J. Kirby, P. R. Heath, P. J. Shaw, and F. C. Hamdy, "Gene expression assays," Advances in clinical chemistry., vol. 44, pp. 247–292, 2007.

[11] J. K. Cowell and L. Hawthorn, "The application of microarray technology to the analysis of the cancer genome," Current Molecular Medicine, vol. 7, no. 1, pp. 103–120, 2007.

[12] G. E. Konecny, B. Winterhoff, and C. Wang, "Gene-expression signatures in ovarian cancer: promise and challenges for patient stratification," Gynecologic Oncology, vol. 141, no. 2, pp. 379–385, 2016.

[13] J. Wan, S. Jiang, Y. Jiang et al., "Data mining and expression analysis of differential IncRNA ADAMTS9–AS1 in prostate cancer," Frontiers in Genetics, vol. 10, p. 1377, 2020.

[14] Y. Bosse and C. I. Amos, "A decade of GWAS results in lung cancer," Cancer Epidemiology, Biomarkers & Prevention, vol. 27, no. 4, pp. 363–379, 2018.

[15] Z. Li, J. Tang, W. Wen et al., "Systematic analysis of genetic variants in cancer–testis genes identified two novel lung cancer susceptibility loci in Chinese population," Journal of Cancer, vol. 11, no. 7, pp. 1985–1993, 2020.

[16] T. P. Lu, C. K. Hsiao, L. C. Lai et al., "Identification of regulatory SNPs associated with genetic modifications in lung adenocarcinoma," BMC Research Notes, vol. 8, no. 1, p. 92, 2015.

[17] T. P. Lu, M. H. Tsai, J. M. Lee et al., "Identification of a novel Biomarker,SEMSA, for non-small cell lung carcinoma in nonsmoking women," Cancer Epidemiology, Biomarkers & Prevention, vol. 19, no. 10, pp. 2590–2597, 2010.

[18] M. E. Ritchie, B. Hipson, D. Wu et al., "limma powers differential expression analyses for RNA-sequencing and microarray studies," Nucleic Acids Research, vol. 43, no. 7, article e47, 2015.

[19] M. Pelizzola, N. Pavelka, M. Foti, and P. Ricciardi-Castagnoli, "AMDA: an R package for the automated microarray data analysis," BMC Bioinformatics, vol. 7, no. 1, p. 335, 2006.

[20] D. Szklarczyk, A. L. Gable, D. Lyon et al., "STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets," Nucleic Acids Research, vol. 47, no. D1, pp. D607–D613, 2019.

[21] P. Shannon, A. Markiel, O. Ozier et al., "Cytoscape: a software environment for integrated models of biomolecular interaction networks," Genome Research, vol. 13, no. 11, pp. 2498–2504, 2003.

[22] G. D. Bader and C. W. Hogue, "An automated method for finding molecular complexes in large protein interaction networks," BMC Bioinformatics, vol. 4, no. 1, p. 2, 2003.

[23] A. Nagy, A. Lánczyk, O. Menyhárt, and B. Györrfy, "Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets," Scientific Reports, vol. 8, no. 1, p. 9227, 2018.

[24] G. K. Smyth, "Linear models and empirical bayes methods for assessing differential expression in microarray experiments," Stat Appl Genet Mol Biol, vol. 3, no. 1, pp. 1–25, 2004.

[25] The Gene Ontology Consortium, "The Gene Ontology project in 2008," Nucleic Acids Research, vol. 36, Supplement 1, pp. D440–D444, 2008.

[26] S. Okuda, T. Yamada, M. Hamajima et al., "KEGG Atlas mapping for global analysis of metabolic pathways," Nucleic Acids Research, vol. 36, no. Web Server, pp. W423–W426, 2008.

[27] X. Jiao, B. T. Sherman, D. W. Huang et al., "DAVID-WS: a stateful web service to facilitate gene/protein list analysis," Bioinformatics, vol. 28, no. 13, pp. 1805–1806, 2012.

[28] B. Snel, G. Lehmann, P. Bork, and M. A. Huynen, "STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene," Nucleic Acids Research, vol. 28, no. 18, pp. 3442–3444, 2000.

[29] Y. Zhou, B. Zhou, L. Pache et al., "Metascape provides a biologist-oriented resource for the analysis of systems-level datasets," Nature Communications, vol. 10, no. 1, p. 1523, 2019.

[30] H. Yan, G. Zheng, J. Qu et al., "Identification of key candidate genes and pathways in multiple myeloma by integrated bioinformatics analysis," Journal of Cellular Physiology, vol. 234, no. 12, pp. 23785–23797, 2019.

[31] S. Couraud, G. Zalcman, B. Milleron, F. Morin, and P. J. Souquet, "Lung cancer in never smokers - A review," European Journal of Cancer, vol. 48, no. 9, pp. 1299–1311, 2012.

[32] Z. Chen, Z. Li, X. Niu et al., "The effect of CYP1A1 polymorphisms on the risk of lung cancer: a global meta-analysis based on 71 case-control studies," Mutagenesis, vol. 26, no. 3, pp. 437–446, 2011.

[33] W. Li, M. Zhang, C. Huang, J. Meng, X. Yin, and G. Sun, "Genetic variants of DNA repair pathway genes on lung cancer risk," Pathology, Research and Practice, vol. 215, no. 10, article 152348, 2019.

[34] S. S. Oh, S. C. Chang, L. Cai et al., "Single nucleotide polymorphisms of 8 inflammation-related genes and their associations with smoking-related cancers," International Journal of Cancer, vol. 127, no. 9, pp. 2169–2182, 2010.

[35] S. Fang, Y. Dai, Y. Mei et al., "Clinical significance and biological role of cancer-derived type I collagen in lung and esophageal cancers," Thoracic Cancer, vol. 10, no. 2, pp. 277–288, 2019.

[36] N. I. Nissen, M. Karsdal, and N. Willumsen, "Collagens and cancer associated fibroblasts in the reactive stroma and its relation to cancer biology," Journal of Experimental & Clinical Cancer Research, vol. 38, no. 1, p. 115, 2019.
[37] D. H. Peng, B. L. Rodriguez, L. Diao et al., “Collagen promotes anti-PD-1/PD-L1 resistance in cancer through LAIR1-dependent CD8+ T cell exhaustion,” *Nature Communications*, vol. 11, no. 1, p. 4520, 2020.

[38] H. Yang, P. Jiang, D. Liu et al., “Matrix metalloproteinase 11 is a potential therapeutic target in lung adenocarcinoma,” *Molecular Therapy Oncolytics*, vol. 14, pp. 82–93, 2019.

[39] R. Kalluri, “Basement membranes: structure, assembly and role in tumour angiogenesis,” *Nature Reviews Cancer*, vol. 3, no. 6, pp. 422–433, 2003.

[40] L. Shen, M. Yang, Q. Lin, Z. Zhang, B. Zhu, and C. Miao, “COL11A1 is overexpressed in recurrent non-small cell lung cancer and promotes cell proliferation, migration, invasion and drug resistance,” *Oncology Reports*, vol. 36, no. 2, pp. 877–885, 2016.

[41] A. S. Lamort, I. Giopanou, I. Psallidas, and G. T. Stathopoulos, “Osteopontin as a link between inflammation and cancer: the thorax in the spotlight,” *Cells*, vol. 8, no. 8, p. 815, 2019.

[42] G. Chakraborty, S. Jain, and G. C. Kundu, “Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms,” *Cancer Research*, vol. 68, no. 1, pp. 152–161, 2008.

[43] Y. Zhang, W. Du, Z. Chen, and C. Xiang, “Upregulation of PD-L1 by SPP1 mediates macrophage polarization and facilitates immune escape in lung adenocarcinoma,” *Experimental Cell Research*, vol. 359, no. 2, pp. 449–457, 2017.

[44] A. Saijo, H. Goto, M. Nakano et al., “Bone marrow-derived fibrocytes promote stem cell-like properties of lung cancer cells,” *Cancer Letters*, vol. 421, pp. 17–27, 2018.

[45] C. Pérez-Ramirez, M. Cañadas-Garre, M. Molina, M. J. Faus-Dáder, and M. Calleja-Hernández, “PTEN and PI3K/AKT in non-small-cell lung cancer,” *Pharmacogenomics*, vol. 16, no. 16, pp. 1843–1862, 2015.

[46] H. Huang, Q. Huang, T. Tang et al., “Differentially expressed gene screening, biological function enrichment, and correlation with prognosis in non-small cell lung cancer,” *Medical Science Monitor*, vol. 25, pp. 4333–4341, 2019.

[47] M. Ni, X. Liu, J. Wu et al., “Identification of candidate biomarkers correlated with the pathogenesis and prognosis of non-small-cell lung cancer via integrated bioinformatics analysis,” *Frontiers in Genetics*, vol. 9, p. 469, 2018.

[48] L. Zhang, R. Peng, Y. Sun, J. Wang, X. Chong, and Z. Zhang, “Identification of key genes in non-small cell lung cancer by bioinformatics analysis,” *PeerJ*, vol. 7, article e8215, 2019.

[49] C. Zhang, M. Jiang, N. Zhou et al., “Use tumor suppressor genes as biomarkers for diagnosis of non-small cell lung cancer,” *Scientific Reports*, vol. 11, no. 1, p. 3596, 2021.

[50] X. Qian, X. Song, Y. He et al., “CCNB2 overexpression is a poor prognostic biomarker in Chinese NSCLC patients,” *Biomedicine & Pharmacotherapy*, vol. 74, pp. 222–227, 2015.

[51] F. Wang, Y. Liu, and Y. Chen, “Pituitary tumor transforming gene-1 in non-small cell lung cancer: clinicopathological and immunohistochemical analysis,” *Biomedicine & Pharmacotherapy*, vol. 84, pp. 1595–1600, 2016.

[52] J. G. Zhao, J. F. Wang, J. F. Feng, X. Y. Jin, and W. L. Ye, “HHIP overexpression inhibits the proliferation, migration and invasion of non-small cell lung cancer,” *PLoS One*, vol. 14, no. 11, article e0225755, 2019.

[53] Z. Jiao, A. Yu, X. He et al., “Bioinformatics analysis to determine the prognostic value and prospective pathway signaling of miR-126 in non-small cell lung cancer,” *Annals of Translational Medicine*, vol. 8, no. 24, p. 1639, 2020.

[54] F. Qiao, N. Li, and W. Li, “Integrative bioinformatics analysis reveals potential long non-coding RNA biomarkers and analysis of function in non-smoking females with lung cancer,” *Medical Science Monitor*, vol. 24, pp. 5771–5778, 2018.