

Genome-Wide Lymphocytic mRNA Sequencing to Identify Epithelial-Mesenchymal Transition Mechanism in Silicosis

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Abstract

Background

Lymphocytes are immune cells that play dual roles in the pathogenesis of silicosis. Epithelial-mesenchymal transition (EMT), a vital phenomenon in the pathogenesis of silicosis, is regulated by cytokines, chemokines, and other molecules secreted by lymphocytes; however, the underlying regulatory mechanism is unclear. Here, we investigated the role of lymphocytes in EMT in silicosis.

Methods

Three patients with silicosis and three healthy controls that underwent pre-job physical examination were recruited; fasting venous blood samples were collected and lymphocytes were separated by Ficoll. High-throughput sequencing technology and bioinformatic analysis were used to identify specific genes and signaling pathways. The results were verified through the detection of related indices of peripheral blood samples.

Results

The baseline characteristics of subjects from silicosis group were matched with those of healthy controls. In comparison with healthy controls, patients with silicosis showed 1915 dysregulated genes that were thought to participate in various biological processes, including angiogenesis, tissue repair, cell proliferation, invasion, migration, and EMT. Protein-protein interaction analysis grouped these genes into three hub targets, including phosphoinositide 3-kinase (PI3K), integrin beta 1 (ITGB1), and integrin-linked protein kinase (ILK). Gene set enrichment analysis (GSEA) confirmed that PI3K, ITGB1, and ILK were tightly associated with EMT through the Wnt signaling pathway, Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathway, and cell adhesion molecular pathway. ITGB1 is a member of the adhesion molecule family. The identified genes were verified through the detection of soluble adhesion molecules in peripheral blood samples of patients with silicosis and healthy subjects.

Conclusion

Dysregulation of PI3K, ITGB1, and ILK in lymphocytes may contribute to EMT via JAK-STAT, Wnt, and cell adhesion molecular pathways in patients with silicosis.

Introduction

Silicosis is an occupational lung disease caused by the long-term inhalation of crystalline silica (SiO_2) dust. It is characterized with persistent inflammation, fibroblast proliferation, and excessive collagen

deposition, eventually leading to pulmonary interstitial fibrosis [1]. Epidemiological studies have indicated silicosis as the primary type of pneumoconiosis with the highest morbidity and mortality in China and other developing countries. A total of 23 million workers are exposed to silica dust each year worldwide [2]. In China, about 3182 workers were diagnosed with silicosis from 2002 to 2016, accounting for 12.7% (95% confidence interval [CI]: 10.8%-14.6%) of all workers exposed to dust [3]. Furthermore, Nanchong, a city located in East China, had a silicosis prevalence of 26.71% and suffered from more severe conditions [4], while Binzhou had a prevalence of 34.02% [5]. These studies suggest that silicosis is more prevalent among silica-exposed populations and has emerged as a serious public health problem threatening the health of relevant practitioners. Therefore, it is necessary to strengthen the understanding about the mechanism underlying silicosis.

Silicotic nodules and diffuse interstitial fibrosis are specific morphological changes related to silicosis, wherein fibrosis is the most significant change in pulmonary interstitium [6]. Most studies suggest that fibrosis is an irreversible process upon initiation of silicosis [7], serving as a landmark event in silicosis. Mechanistically, over-proliferation and differentiation of fibroblasts contribute to pulmonary fibrosis, a complex biological process [8-13]. Epithelial-mesenchymal transition (EMT) was recently shown to play a crucial role in pulmonary fibrosis [6]. EMT refers to the phenomenon characterized with the loss of polarity of epithelial cells and the disappearance of the tight junction between cells, resulting in their resemblance to mesenchymal cells in terms of morphology and characteristics [14].

Inhaled silica dust particles get deposited in the lung tissues and may be engulfed by macrophages, resulting in the induction of an immune response, followed by the initiation of apoptosis and the release of inflammatory cytokines. The consequence includes EMT and even fibrosis of pulmonary tissues. Lymphocytes in human peripheral blood play dominant roles in immune response. Thus, the analysis and interpretation of genetic materials in lymphocytes may reflect the process underlying the development of silicosis.

The rapid development in the field of molecular biology and computer science allows investigation of the molecular mechanisms underlying silicosis at the genome-wide level [15]. Here, we identify the role of lymphocytes in EMT and investigate the underlying molecular mechanism in silicosis using high-throughput sequencing and bioinformatic tools. We validate the identified genes with the physical examination of samples.

Material And Methods

Objectives

Three patients with silicosis and three healthy controls that underwent pre-job physical examination at the Xinxiang Occupational Disease Prevention and Control Hospital from September to October 2018 were recruited (Table 1). The two groups were matched by age, sex, body mass index (BMI), and other

baseline characteristics. Inclusion criteria of silicosis were in line with the diagnostic criteria of pneumoconiosis (Diagnostic Criteria of Pneumoconiosis (GBZ 70-2015) as follows: patients diagnosed at Xinxiang Occupational Disease Prevention and Treatment Hospital without active pulmonary tuberculosis or extrapulmonary tuberculosis; without complications other than pulmonary infection, chronic obstructive pulmonary disease, and pulmonary heart disease; and without severe heart, liver, and kidney diseases. Informed consent was provided by all participants. Exclusion criteria were as follows: subjects that failed to meet the inclusion criteria; patients with psychiatric problems; those with severe heart, liver, or kidney diseases; patients with poor compliance that failed to cooperate with the study criteria. The healthy controls were confirmed to have no physical disorders, which may affect the lymphocytes in the peripheral blood.

Table 1
Detailed information of subjects involved in this study

Sample	Group	Age	Duration of dust exposure (year)	Diagnosis	BMI (kg/m ²)
T1	Healthy control	40	0	0	25.9
T2	Healthy control	61	0	0	25.7
T3	Healthy control	55	0	0	24.5
T4	silicosis	43	13	□	25.2
T5	silicosis	60	15	□	25.6
T6	silicosis	58	18	□	24.8

Note: BMI = weight/height squared

Isolation of lymphocytes

In brief, 5 mL of fresh blood samples were collected from every subject after 12 h of fasting. All blood samples were treated with the anticoagulant ethylenediaminetetraacetic acid (EDTA), followed by dilution with an equal volume of phosphate-buffered saline (PBS). The samples were centrifuged at 500-1000 ·g for 20-30 min at room temperature(20±5°C). The middle layer was transferred to another tube and resuspended in 10 mL PBS. The mixture was centrifuged twice at 250 ·g for 10 min and the pellet was resuspended and used for further analysis.

Total RNA extraction and high-throughput sequencing

Total RNA was extracted from lymphocytes using the blood total RNA isolation kit according to the manufacturer's instruction. RNA concentration was quantified using a microplate reader. The extracted RNA was amplified using polymerase chain reaction (PCR). Sequencing library was constructed using 150 ng of each recovered PCR product, and the library was analyzed on an Illumina MiSeq platform by Generry Inc. (Shanghai, China).

Identification of differentially expressed genes (DEGs)

Gene expression values obtained from high-throughput sequencing were normalized as transcript per million. Boxplot was used to assess the distribution of the detected genes in each sample. The limma test was employed to identify the DEGs between silicosis and control groups. Genes satisfying the following criteria were considered as DEGs: $|\log_2(\text{fold change})| \geq 1$, adjusted P-value < 0.05.

Functional annotation of DEGs by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

DEGs identified were subjected to Cytoscape, an analysis tool suitable for genome-wide integrated discovery and annotation that provides typical batch annotation. KEGG (<https://www.kegg.jp/>), one of the most popular biological information databases, aims to determine advanced functions in biological systems. At the molecular level, KEGG specifically integrates a large number of practical program database resources from high-throughput experimental technologies. GO is a widely used ontology based on three aspects, namely, cellular component (CC), molecular function (MF), and biological process (BP). Annotation items with a P-value less than 0.05 were selected for further investigation.

Construction of protein-protein interaction (PPI) network and screening of hub genes

The Interactive Gene Retrieval Search Tool was used to construct the PPI network, a visual analytical platform for comprehensive gene expression profiling and meta-analysis at the protein level (available online at <https://www.networkanalyst.ca/>). Molecular Complex Detection (MCODE, version 1.5.1), a plugin of Cytoscape, was employed to investigate the hub genes based on the PPI network. The criteria for MCODE analysis were as follows: degree cutoff value, 2; MCODE scores, > 5; max depth, 100; k-score, 2; node score cutoff, 0.2. Genes with a degree cutoff value ≥ 10 were identified as hub genes, which were submitted for GO and clustering analysis using OmicShare (<http://www.omicshare.com/tools>), an open data analysis platform.

Gene set enrichment analysis (GSEA)

GSEA is a computational method that determines whether a prior defined set of genes shows statistically significant concordant difference between two biological states. Genes involved in Wnt signaling pathways, Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathways, and cell adhesion molecules were employed to investigate the regulatory role of phosphoinositide 3-kinase (PI3K), integrin beta 1 (ITGB1), and integrin-linked protein kinase (ILK) in these signaling pathways. A GSEA map was employed to assess the enrichment efficiency of these models.

Verification of identification results

89 cases of silicosis diagnosed at the Occupational Disease Prevention and Treatment Center of a coal industry group from 2013 to 2015 were selected as the case group (all tunneling workers). In addition, 94 workers exposed to dust with normal chest radiograph were selected as the control group (all tunneling workers) from nine coal mines. Enzyme-linked immunosorbent assay (ELISA) was used to detect the

expression levels of soluble adhesion molecules (SICAM) in the serum samples of subjects. The verification of results was undertaken for all the samples obtained.

Statistical analysis

SPSS version 24.0 was used for statistical analysis. Baseline characteristics were presented as mean \pm standard deviation (SD) for parametric indicators, and differences between silicosis patients and healthy controls were compared using the *t*-test. Fisher's exact test was used to investigate differences in different silicosis stages. A P-value less than 0.05 was considered statistically significant.

Results

Baseline characteristics

We included six subjects in this study, three of which were diagnosed with silicosis. The average age was 52.0 ± 10.8 years (range, 40 to 61), and the exposure period to dust ranged from 13 to 18 years. Other three subjects without dust exposure history were recruited as healthy controls; their average age was 53.7 ± 9.3 years (range, 43 to 60). There was no difference in age between silicosis and control groups ($t = 0.20$, $P = 0.849$). The difference in BMI was not statistically significant (BMI, 25.4 ± 0.8 versus 25.2 ± 0.2 , $t = 0.34$, $P = 0.753$). Thus, the differences in baseline characteristics among two groups were excluded, and the patients could be used for genome-wide profiling and investigation.

Identification of differentially expressed mRNAs (DEmRNAs) in silicosis

We separated lymphocytes from venous blood samples and used for genome-wide mRNA profiling. A total of 80813 genes were identified, of which 53169 mRNAs have been previously reported and 10614 mRNAs were predicted to be new. We used the violin diagram and correlation analysis to assess the quality of profiling data. As shown in Figure 1, the normalized data of all samples showed high homogeneity in median and quartile range, and no outliers were found. Correlation analysis revealed the high correlation between groups, but the samples from different groups showed a relatively low correlation strength. We performed the limma test between silicosis and control groups and identified 1915 DEmRNAs; 987 of 1915 mRNAs were upregulated, while 928 mRNAs were downregulated.

Functional annotation of DEmRNAs

To investigate the potential role of these DEmRNAs in the pathogenesis of silicosis, we performed GO and KEGG analyses and found their involvement in biological processes of embryogenesis, angiogenesis, tissue repair, immune response, cell survival, proliferation, apoptosis, cell invasion, cell migration, EMT, and pulmonary fibrosis (Figure 2). KEGG analysis highlighted signaling pathways, including JAK-STAT, Wnt, and focal adhesion, that were mainly involved in silicosis initiation.

PPI network construction and module analysis

To investigate the interaction between DEmRNAs at the protein level, those involved in JAK-STAT, Wnt, and focal adhesion pathways were used to construct a PPI network (Figure 3). Consistent with the KEGG enrichment analysis, PPI network showed three functional groups of JAK-STAT signaling pathway, Wnt signaling pathway, and cell adhesion molecules that were associated with the pathogenesis of silicosis. DEmRNAs with an enrichment score > 0.6 were exported and used to construct modules in Cytoscape. After network fitting analysis, three genes, including PI3K, ITGB1, and ILK, were identified as the hub genes that may play a dominate role in the pathogenesis of silicosis.

Identification of the signaling pathways related to the hub genes in silicosis

The signaling pathways derived from the KEGG enrichment analysis were used to investigate the role the PI3K, ITGB1, and ILK in the initiation of silicosis. As shown in Figure 4, 30 signaling pathways were found to simultaneously involve PI3K, ITGB1, and ILK. Of these pathways, JAK-STAT signaling pathway, Wnt signaling pathway, and cell adhesion molecules were identified with the lowest P-value.

Targeted analysis of the signaling pathways involving the hub genes

GSEA provides a completely new algorithm for the identification of the mRNAs of interest. Here, we employed PI3K, ITGB1, and ILK as well as JAK-STAT signaling pathway, Wnt signaling pathway, and cell adhesion molecules to assess the regulatory relationship. As shown in Figure 5, the enrichment scores of PI3K, ITGB1, and ILK toward JAK-STAT signaling pathway, Wnt signaling pathway, and cell adhesion molecules were all statistically significant, indicating that PI3K, ITGB1, and ILK may contribute to the pathogenesis of silicosis through JAK-STAT, Wnt, and cell adhesion molecules.

Verification of identification results

To verify the identification results, our team have selected 89 patients with silicosis as the case group and 94 workers exposed to dust with normal chest X-ray as the reference. The social-demographic characteristics and occupational history of subjects were investigated, and the expression levels of SICAM in the peripheral blood were detected (Table 2).

Table 2
Basic characteristics of the two groups of subjects

Characteristics	Control (94)	Case (89)	U (χ^2)	P
Type of work	Tunneling workers	Tunneling workers		
Age	44.6 ± 10.3	64.8±10.3	15.29	< 0.0001
Working year	20.7 ± 11.5	28.2 ± 7.2	5.37	< 0.0001
Smoking rate	42.6%	37.1%	0.5714	0.4497
Smoking index	151.7 ± 205.0	260.4 ± 280.0	2.98	0.0033
SICAM	1461.5 ± 245.03	1685.1 ± 143.32	7.58	< 0.0001

Table 2 shows that SICAM levels were significantly different between the case and control group ($U = -7.58$, $P < 0.0001$). However, age, length of service, and smoking index were not balanced between the two groups. We conducted a generalized linear model analysis and found that SICAM levels were still significantly different between the two groups ($\chi^2 = 15.837$, $P < 0.0001$) after adjustment for the three confounding factors (Table 3).

Table 3
Analysis of results of generalized linear model

Parameter	β	Standard error	95% Wald		Hypothesis test		
			Lower CL	Upper CL	Chi square	df	Sig
Intercept	1554.257	112.000	1334.748	1773.766	192.591	1	0.000
Group	-182.902	45.960	-272.981	-92.823	15.837	1	0.000
Age	2.343	2.0369	-1.650	6.335	1.323	1	0.250
Working years	-0.490	1.919	-4.251	3.270	0.065	1	0.798
Smoking index	-0.027	0.061	-0.147	0.093	0.197	1	0.657

The receiver operating characteristic (ROC) curve was used to validate the diagnostic value for silicosis using serum SICAM level. The area under the curve of 0.762 and a cutoff value of 1487.91 pg/mL provided the optimum diagnostic performance with a sensitivity and specificity of 94.4% and 55.3%, respectively (Figure 6).

Discussion

While the incidence of pneumoconiosis is decreasing every year worldwide [16–19], the number of pneumoconiosis cases is still high in China [20–24]. Silicosis is the main type of pneumoconiosis, accounting for about 90% of all occupational diseases [25]. Studies by Wang Dan and others show that the data reported on occupational diseases is less than 7% of the actual incidence [26]. Silicosis is thought to emerge as one of the major occupational diseases that seriously affects the health of concerned populations.

The pathogenesis of silicosis is complex. The entry of silica dust into the body results in macrophage damage in the early stage, followed by the triggering of the immune system, formation of antigen-antibody immune complexes, and development of pathological changes related to silicosis. Lymphocytes in the human peripheral blood are important cellular components of immune response. The analysis and interpretation of genetic material in lymphocyte nucleus may reflect the genesis and development of silicosis. The occurrence of silicosis is affected by several targets. Therefore, it is essential to explore the mechanism underlying silicosis and identify the key reliable targets at the molecular level.

The recent development in biogenetic exploration technologies has facilitated the understanding of DEGs in several diseases to provide ideas for accurate treatment [24, 27, 28]. For instance, four molecular markers associated with the development of gliomas have been identified through biogenetic approaches [24]. A study on hepatocellular carcinoma employed the microarray technology to search and analyze a biological database and finally identified 16 hub genes closely related to the development of hepatocellular carcinoma [27]. Another study analyzed the expression levels of 322 immune genes and revealed the enhancement in the immune characteristics of patients with neuroblastoma; this study finally selected eight immune genes as the molecular markers of neuroblastoma [28]. Biotechnology and its application in disease-related gene prediction may provide new cues and support for the selection of DEGs related to silicosis.

In the present study, we used bioinformatic tools to identify the DEGs and target molecular markers from peripheral blood lymphocytes. GEO2R for the analysis of 80813 whole gene transcriptome data sets may help us identify the DEGs between silicosis and non-silicosis samples. With enrichment analysis, we found that these DEGs were mainly associated with JAK-STAT, Wnt, and adhesion molecule signaling pathways with respect to EMT. Among the most important modules of the PPI network, three central genes (PI3K, ITGB1, and ILK) showed the highest score, suggestive of their important roles in the occurrence or development of EMT in silicosis.

ITGB1 is a member of the integrin family, while integrin is a member of the adhesion molecule group [29]. Therefore, we detected the expression levels of SICAM in the serum samples from 89 silicosis patients and 94 dust-exposed workers, and found that the difference was statistically significant. After adjustment for age, length of service, and smoking index, the expression levels of SICAM in the serum were still statistically significant between the two groups. The results of ROC curve analysis also suggested that

SICAM could be used as an index for the early screening of silicosis. Thus, PI3K, ITGB1, and ILK may play an important role in the occurrence and development of EMT in silicosis.

EMT refers to the loss of polarity of epithelial cells and the disappearance of tight junctions between cells under the effect of certain factors, thereby imparting the morphology and characteristics of interstitial cells [14]. The process of pulmonary fibrosis may be regarded as EMT in tissue repair. The level of E-cadherin protein and the relative expression of E-cadherin gene in silicosis model group decreased, while the levels of alpha smooth muscle actin and vimentin proteins and the relative expression of their RNAs increased, suggestive of the occurrence of EMT during the development of silicosis [30–32].

PI3K is a very important member of the phospholipid kinase family. It has lipid kinase and protein kinase activities. P13K signaling pathway is an important pathway widely involved in cell proliferation, differentiation, cycle regulation, and apoptosis. Activated PI3K may produce the second messenger phosphatidylinositol (3, 4, 5)-trisphosphate (PIP3) and further phosphorylate protein kinase B (AKT) protein. Activated AKT enters the nucleus and induces or inhibits the downstream proteins for the regulation of cell survival and proliferation, apoptosis, angiogenesis, and pulmonary fibrosis [33–35]. Studies have confirmed that the inhibition of PI3K/AKT signaling pathway may significantly reduce hepatic stellate cell (HSC) proliferation, alpha-collagen type III mRNA expression, and collagen type III secretion [36]. Over-activation of P13K/AKT/hypoxia-inducible factor-1a (HIF-1a) pathway may affect the normal repair of alveolar epithelial cells through the regulation of cell proliferation and apoptosis, leading to the production of collagen III and formation of pulmonary fibrosis [37]. Some studies have shown that HIF-1a may be regulated by Snail and beta-catenin signaling pathways to promote the transformation of alveolar epithelial cells to interstitial cells and participate in the formation of pulmonary fibrosis [38]. These results suggest that the activation of P13K signaling pathway is associated with the process of pulmonary fibrosis via EMT.

ITGB1 is a member of the integrin family of proteins that are mainly involved in the regulation of cell adhesion and recognition during embryogenesis, hemostasis, tissue repair, immune response and tumor cell metastasis [29].

As a member of the cell adhesion molecule family, integrins not only function as adhesion molecules but also are also involved in transmembrane connection. These molecules can connect the extracellular matrix-integrin molecule-cytoskeleton protein and participate in the activation of multiple signal transduction pathways. Integrins play an important role in several physiological and pathological processes. The connection of the extracellular matrix with integrin may result in the formation of focal adhesion (FAP). Many cytoskeletal proteins are expressed in FAP, including fibronectin (FN), laminin (LN), collagen, and wavin (VN). In addition, many important signal transduction molecules such as FAK, ILK, and PI3K could be activated by the extracellular matrix and integrin, subsequently leading to the activation of a variety of downstream signal transduction pathways regulating cell proliferation, survival, and differentiation.

Integrin is thought to be closely related to the occurrence of EMT. The abnormal secretion of integrin in response to trauma-induced tissue stimulation may promote EMT and development of fibrosis [39]. Galliher et al. found that integrin $\alpha_v\beta_3$ promotes EMT by regulating the expression of transforming growth factor-beta (TGF- β) receptor. In the absence of integrin β_3 , TGF- β may not induce EMT [40]. Thus, integrin is an upstream molecule that promotes EMT.

ILK is a serine/threonine protein kinase that binds to the cytoplasmic domain of integrin β_1 and β_3 subunits and participates in integrin, growth factor, Wnt, and TGF- β /Smad signal transduction pathways. ILK also regulates cell growth, survival, cell cycle, EMT, apoptosis, invasion, migration, cancer, and angiogenesis.

Studies have shown that ILK is closely related to EMT of cells and promotes the occurrence and development of EMT. Serrano et al. found that EMT in mammary epithelial cells results in a significant increase in the expression of ILK; this transformation process may be effectively reversed by blocking ILK expression [41]. Mao Zhongyi et al. found that the expression of ILK and TGF- β 1 increased with the development of liver fibrosis and positively correlated with the degree of liver fibrosis [42]. Li Xiaoqin and others confirmed that ILK was closely related to the occurrence and development of renal interstitial fibrosis, and that its expression may serve as an important reference index to judge the progression of renal diseases and prognosis [43]. Cui Hongshuai's research shows that the level of ILK increases with the severity of pulmonary fibrosis in mice, suggestive of the close relationship between ILK and occurrence of pulmonary fibrosis [44]. ILK may reduce the degradation of β -catenin and its transfer to the nucleus. It binds to T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) in the nucleus and promotes the transcription of target genes, thereby promoting EMT [45]. Thus, the activation of ILK is closely related to EMT.

Our study has some limitations. First, the screening of the three key genes related to the development and progression of silicosis is based on bioinformatic technology, which needs further verification with in vitro and in vivo experiments. Collection of samples from silicosis and non-silicosis subjects and establishment of a suitable animal model of silicosis are warranted for the verification of the mechanism of action of the three genes in lymphocytes. Second, the data set of the transcription group is based on the lymphocytes from three cases of silicosis and three control subjects. The data were collected thrice and analyzed without mixing to obtain maximum and minimum values; the minimum values were used. In the second phase, we plan to re-derive the transcription group data set according to the conventional method to verify the similarities and differences between the two detection methods.

Conclusions

Dysregulation of PI3K, ITGB1, and ILK in lymphocytes may contribute to EMT via JAK-STAT and Wnt signalling pathways in silicosis.

Declarations

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Authors' contributions

Conceptualization, Haibin Li and Sanqiao Yao; Methodology, Lin Zhang, Yingzheng Zhao, Yi Guan; Investigation, Haibin Li, Ning Li; Software, Haibin Li; validation, Haibin Li; data curation, Haibin Li, Nan Liu, Hao Xu, Zhihao Lv, Yongheng Wang, Xueze Si; writing—original draft preparation, Haibin Li; writing—review and editing, Sanqiao Yao and Haibin Li; supervision, Haibin Li and Weidong Wu; project administration, Sanqiao Yao; Manuscript submission, Haibin Li. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate all subjects signed an informed consent before being enrolled. The study was approved by the Ethics Committee of the Xinxiang Medical University

(protocol number XYLL-2017086, approved 3 March 2017).

Competing interests

The authors declare that they have no competing interests

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Figures

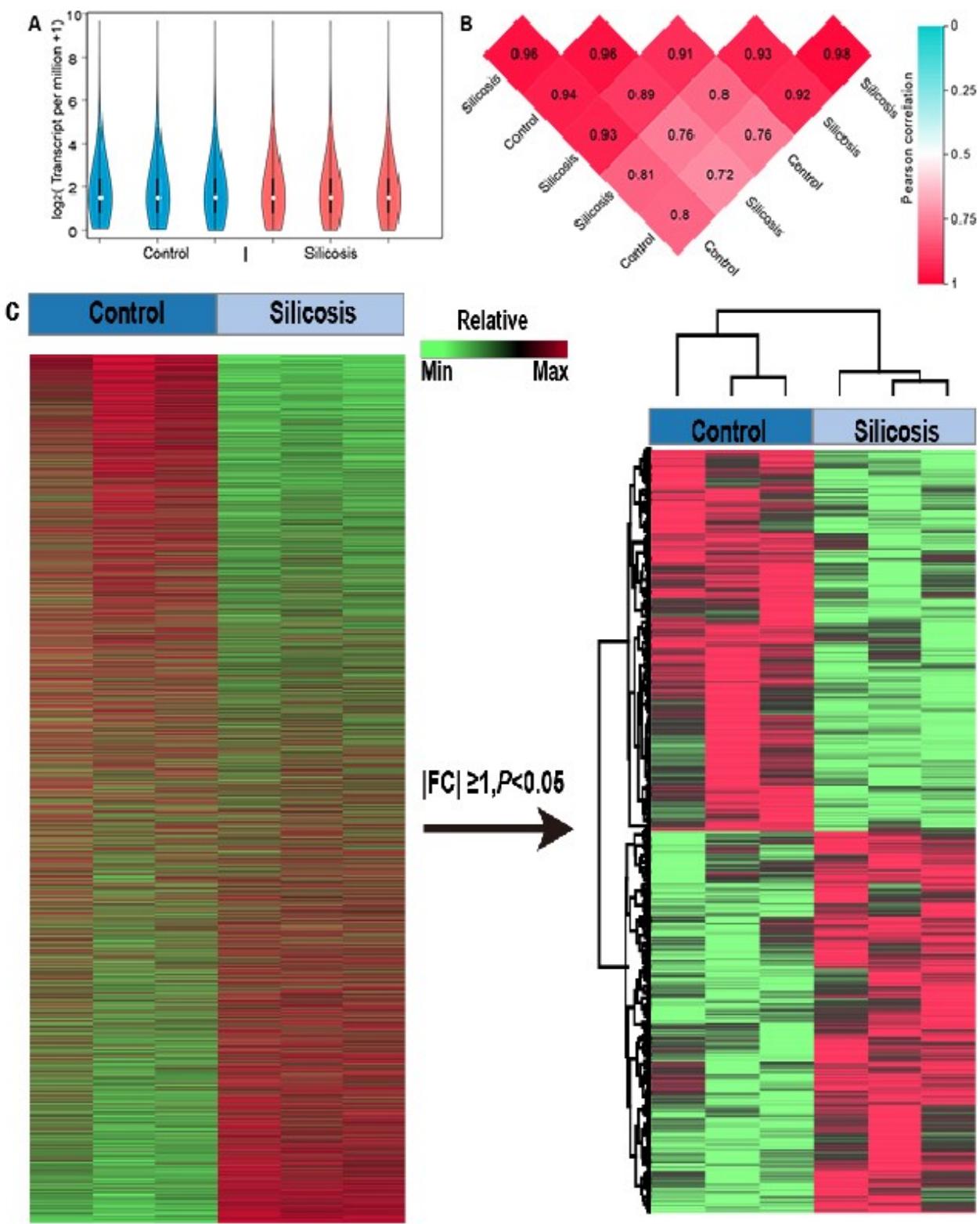


Figure 1

Identification of differentially expressed mRNAs in silicosis. A. Boxplot showing the distribution of genome-wide profiling data in each subject. B. Correlation analysis of profiling data in each subject. C. Heat map of genome-wide profiling datasets. Red, upregulation; Green, downregulation.

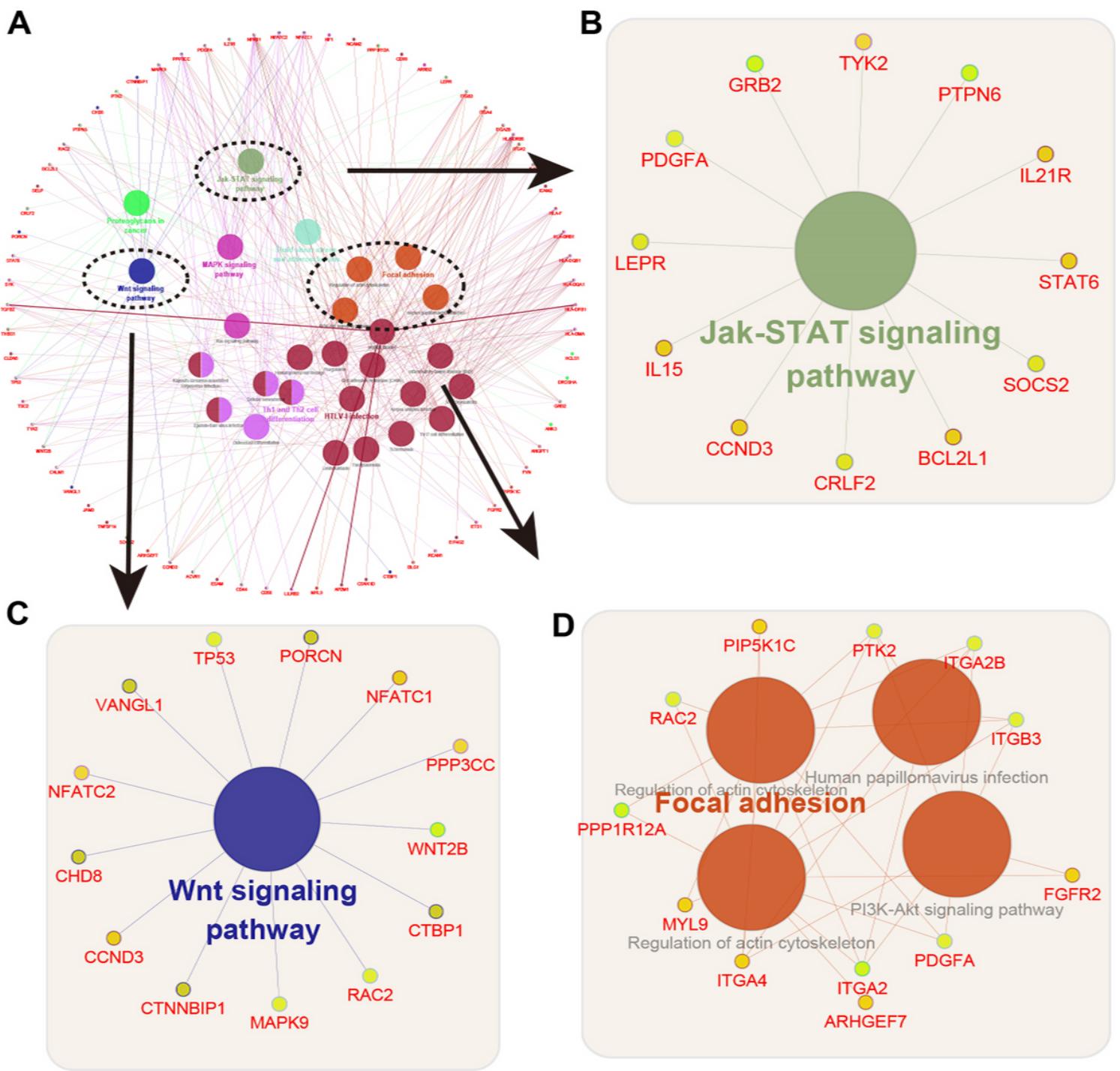
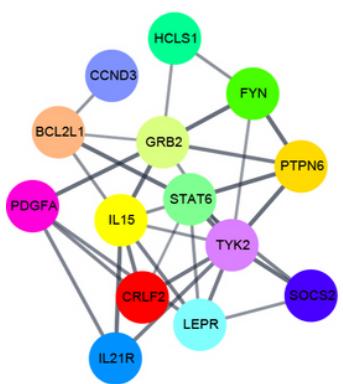


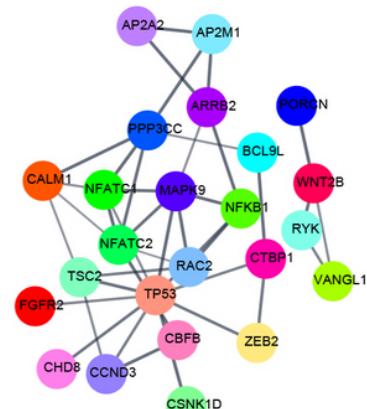
Figure 2

Functional annotation of differentially expressed mRNAs (DEmRNAs) in silicosis. A. Functional annotation and enrichment of DEmRNAs in silicosis; functional groups of interest were labeled using black circles. B-D. Signaling pathways or functional groups identified in A.

Jak-STAT signaling pathway



Wnt signaling pathway



Cell adhesion molecules

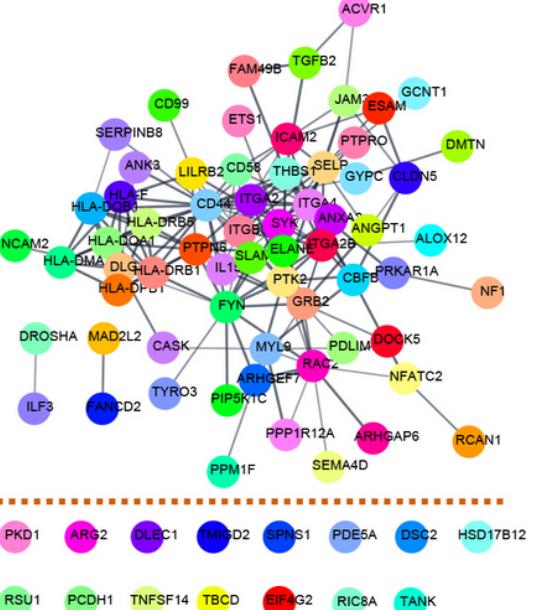


Figure 3

Protein-protein interaction (PPI) network in silicosis. Different networks were separated using vertical black dotted lines; proteins under the orange dotted line were differentially expressed at the mRNA level but did not fit into the PPI network.

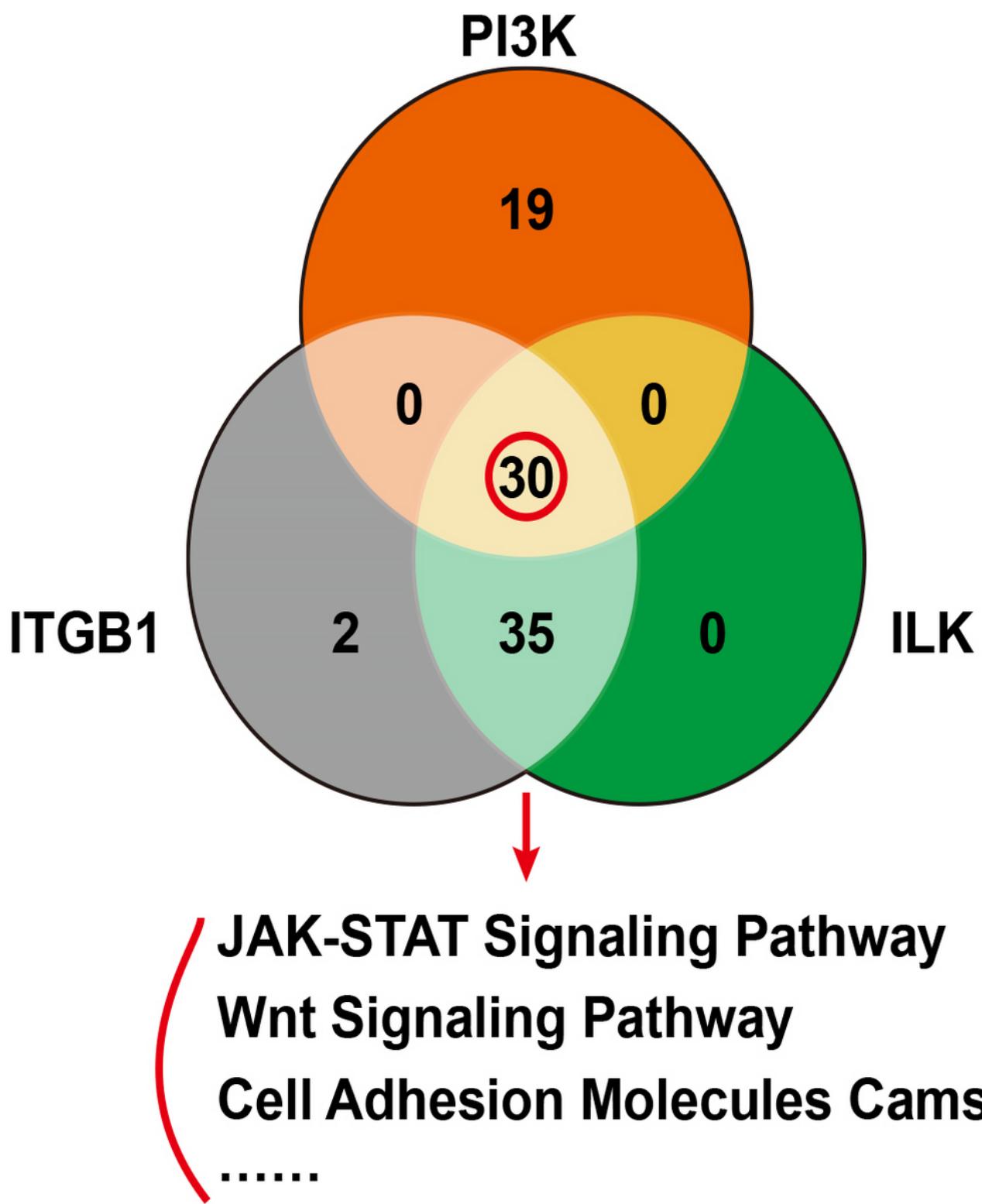
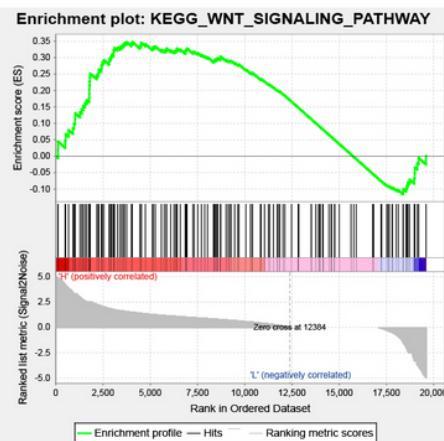


Figure 4

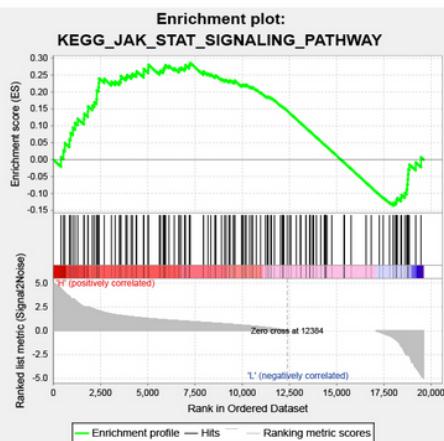
Venn diagram showing the signaling pathways simultaneously involving PI3K, ITGB1, and ILK.

PI3K

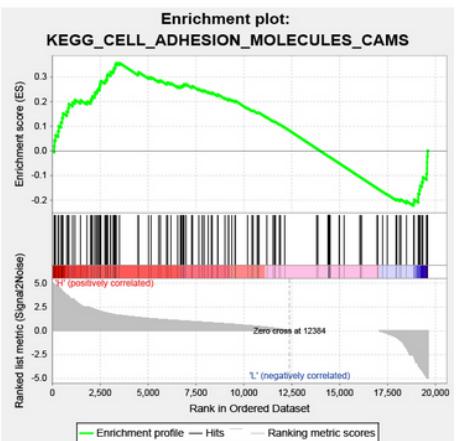
Wnt signaling pathway



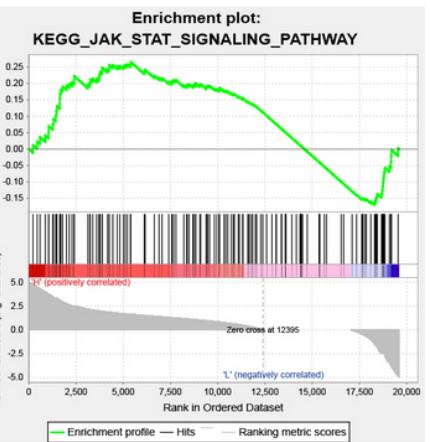
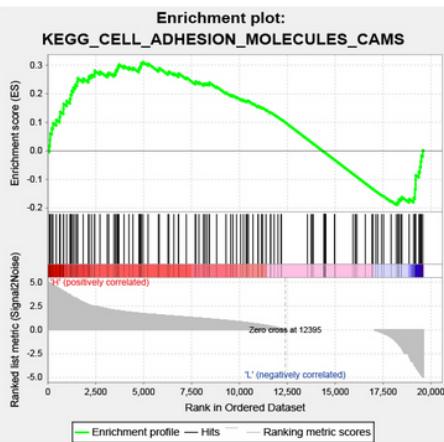
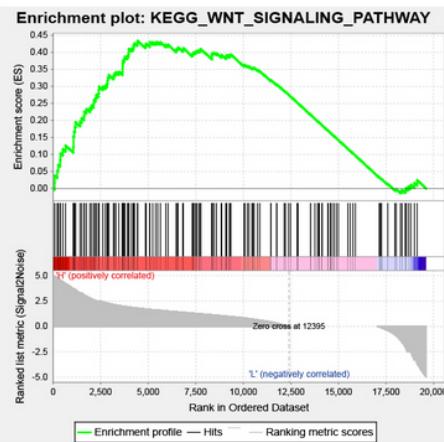
Jak-STAT signaling pathway



Cell adhesion molecules



ITGB1



ILK

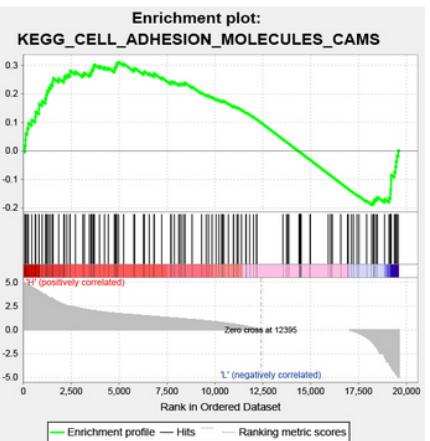
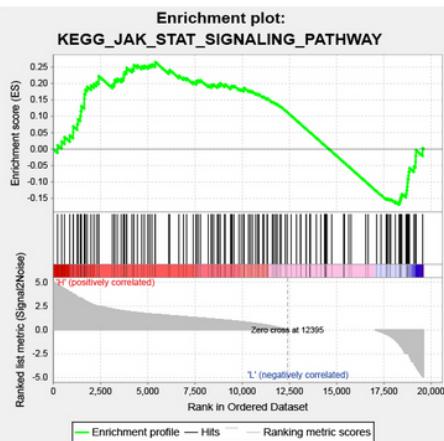
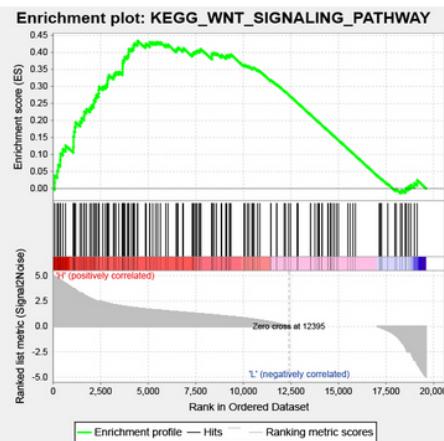


Figure 5

Targeted gene set enrichment analysis of PI3K, ITGB1, and ILK in silicosis.

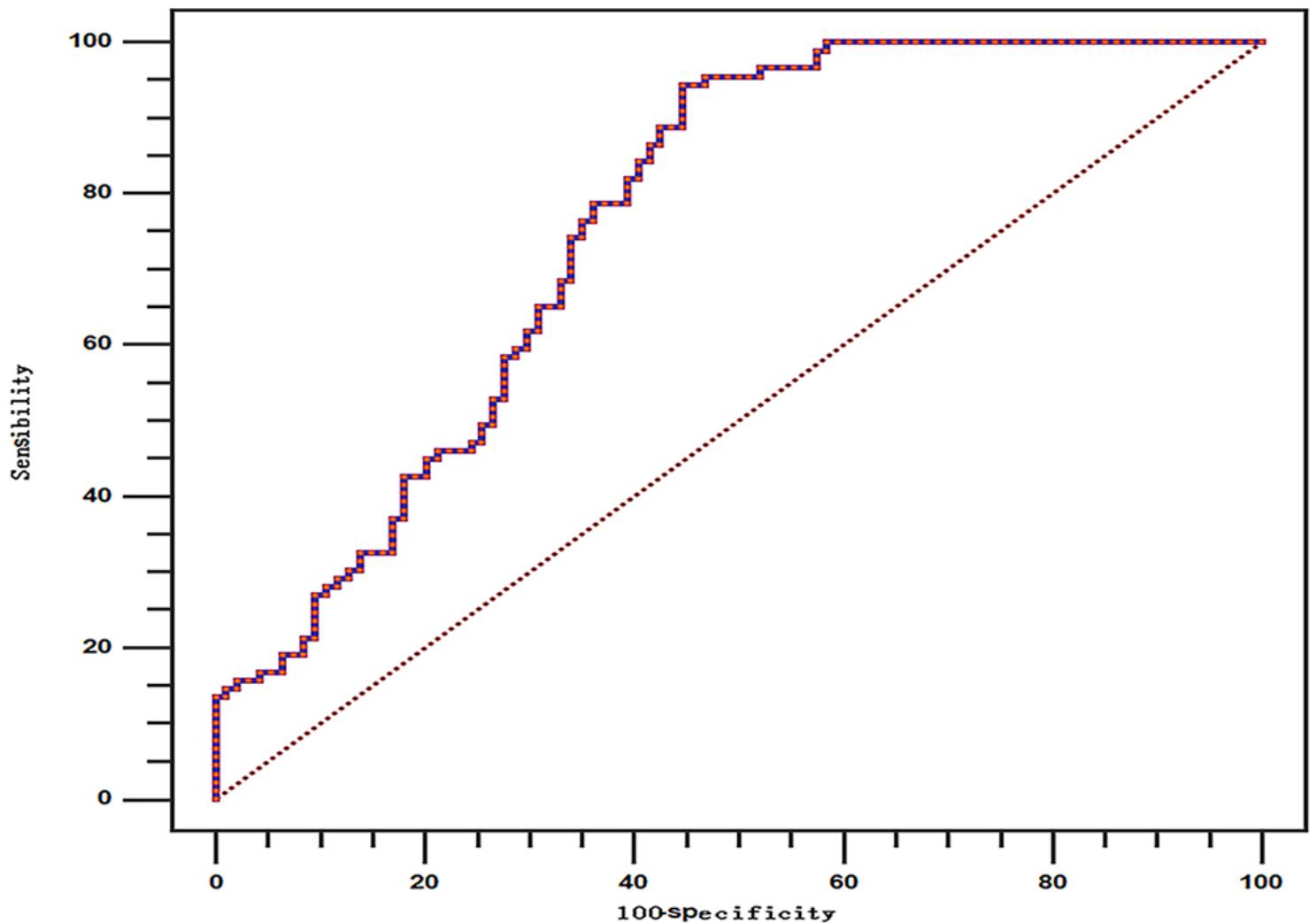


Figure 6

Screening ROC curve of silicosis by SICAM.