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Differential expression and functional analysis of lung cancer gene expression datasets: A systems biology perspective

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Abstract. There is an inherent need to identify differentially expressed genes (DEGs), characterize these genes and provide functional enrichment analysis to the publicly available lung cancer datasets, primarily coming from next-generation sequencing data or microarray gene expression studies. The risk of lung cancer in patients with smokers is manifold, and with chronic obstructive pulmonary disease (COPD) it is 2- to 5-fold greater, compared with smokers without COPD. In the present study, differential expression analysis and gene functional enrichment analysis of lung cancer gene expression datasets obtained from NCBI-GEO were performed. The result identifies a significant number of DEGs which have at least a 2-fold change in their expression. Among them, six genes were found to have a 4-fold change in the expression level, and 47 genes exhibited a 3-fold change in the expression. It was also observed that most of the genes were upregulated and few genes were downregulated.

Introduction

Lung cancer is one of the most common cancer types occurring in both men and women. According to the American Institute for Cancer Research (AICR), approximately 2 million new cases of lung cancer were reported in the year 2018 (1,2). As per the GLOBOCAN report of 2018, lung and breast cancer have the highest incidence rate, with lung cancer (Fig. 1) being the leading cause of mortality (2) consistent with other reports (3,4,5). A list of the top 20 countries with the highest rate of lung cancer in 2018 is presented in Fig. 2 (1). Strong evidence suggests that arsenic-containing drinking water and high-dose of beta-carotene augment the risk of lung cancer. In addition, consuming red meat and alcoholic may increase the risk (6). Lung cancer begins in the lungs as a mutation in oncogenes and proliferates as primary tumor and may spread to lymph nodes or other organs in the body by metastases. It is classified as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Of the two, NSCLSC accounts for approximately 85% among all the lung cancer cases. The major subtypes of NSCLC are adenocarcinoma (40%), squamous cell carcinoma (30%), and large cell carcinoma (15%) (7). Smoking is the main causative agent of lung cancer. For a non-smoker, exposure to passive smoking also causes lung cancer. In general, exposure to a carcinogen increases the risk of developing lung cancer, which includes asbestos, arsenic, chromium, nickel, radon, tobacco, benzene, cadmium, formaldehyde and crystalline silica (8). It has been reported that there is approximately 16% chance for 5-year survival (9).

As far as lung cancer is concerned, the chronic obstructive pulmonary disease (COPD) is a significant risk factor which can be associated with the patient's susceptibility to cigarette smoking. In fact, severe inflammation induced due to toxic gases trigger COPD and lung cancer (10). The most common COPD are emphysema and chronic bronchitis. Bronchitis is inflammation of the bronchi. Emphysema causes damage to the alveoli, the air sacs in the lungs. The walls of the damaged alveoli become stretched out and make it difficult for diffusion. COPD is primarily caused by smoking and long-term exposure and contact with harmful pollutants that include certain chemicals, dust, or fumes and rarely, by alpha-1-antitrypsin and deficiency or a genetic condition.

COPD is measured by spirometry grading systems and one of them is GOLD classification. The GOLD classification is used for determining COPD severity and helps in prognosis and treatment plan. Based on spirometry testing, COPD and is graded as: mild (grade 1), moderate (grade 2), severe (grade 3) and very severe (grade 4). It is dependent on the result of the spirometry test of a patient's FEV1, i.e., the volume of air one may breathe out of the lungs in the first one second of a forced expiration. As FEV1 decreases, the severity increases. With the progress in time, the patient is more susceptible to various complications, including respiratory infections, heart problems, high blood pressure in lung arteries (pulmonary hypertension), flu, colds, pneumonia, depression, anxiety, and lung cancer.

In fact, COPD and lung cancer are linked in a number of ways, one being that smoking is the most common risk

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factor; others include passive smoke or exposure to chemicals or other fumes in the workplace. It has been estimated that between 40 and 70% of individuals with lung cancer also have COPD and it is concluded that COPD is a risk factor for lung cancer (11,12). By contrast, a study by Durham and Adcock (9) suggested that COPD is a driving factor in lung cancer. COPD is the leading cause of mortality projected to rank 3rd in 2020 (13) and comes under the environmental factors such as smoking (14). Exacerbation of COPD exhibits various symptoms that include cough, production of sputum or shortness of breath. It can be caused either by bacterial or viral infections or inhaled particles. The genetic factor can also be helpful in determining the frequency of this disease (15).

Gene expression studies are an important tool for transcriptomic analysis of an organism that helps to quantify expression level genes in both disease and normal conditions. Gene expression profiles of two different conditions (disease versus normal) can be compared to reveal potential key regulators or differentially expressed genes (DEGs), or co-regulated genes, either up- or downregulated (16). The key regulators or DEGs may be possible gene biomarker responsible for the disease condition (17,18). A few gene expression studies on COPD and lung cancer (14,15) are available; however, our aim is to identify DEGs and determine their functional analysis. The present study presents a systems biology perspective to decipher DEGs in lung cancer using microarray gene expression profiles and determine their functional analysis.

Materials and methods

Datasets. In order to identify DEGs, i.e., key gene biomarkers, two types of samples with multiple replicas were required: lung cancer tissue samples and healthy lung tissue samples. On studying these samples, factors that could be the reason for COPD or lung cancer were identified. These factors were genetic or environmental. COPD may be an emphysema type. In emphysema, air sacs are damaged and the patient does not get the oxygen required. Exacerbation of COPD can be diagnosed on the basis of symptoms including cough, shortness of breath, and generation of sputum.

In the present study, publicly available gene expression profiles were obtained from Gene Expression Omnibus (GEO accession no. GSE1650) where data referable to patients were properly anonymized by submitters and informed consent was obtained by the investigators during the original data collection. The following information labels were available and collected for each sample: sample GSM number, status (public on month/day/year), title (number letter) sample type (RNA), source name (lung tissue), organism (Homo sapiens), extracted molecule (total RNA), and description (lung tissue and resected lung taken from smokers).

Of the 30 patients, 18 samples belong to severe emphysema patients and the remaining 12 samples belong to patients having mild or no emphysema. A comparison was made of the expression profiles of severely emphysematous tissue and normal/mildly emphysematous lung tissue from smokers with nodules suspicious of lung cancer. The comparison provides insights into the pathogenetic mechanisms of COPD.

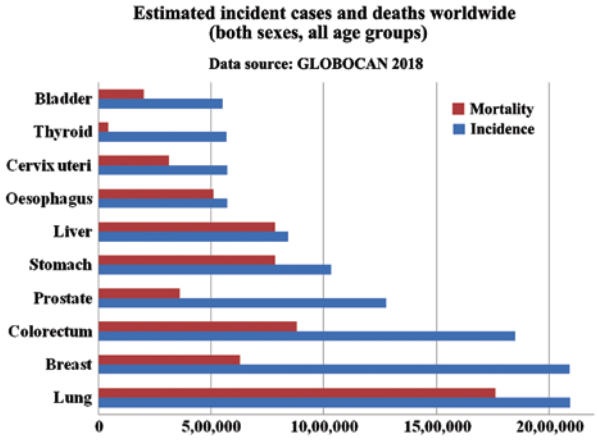

Figure 1. Estimated incident cases and deaths for the top 10 cancer types (2).

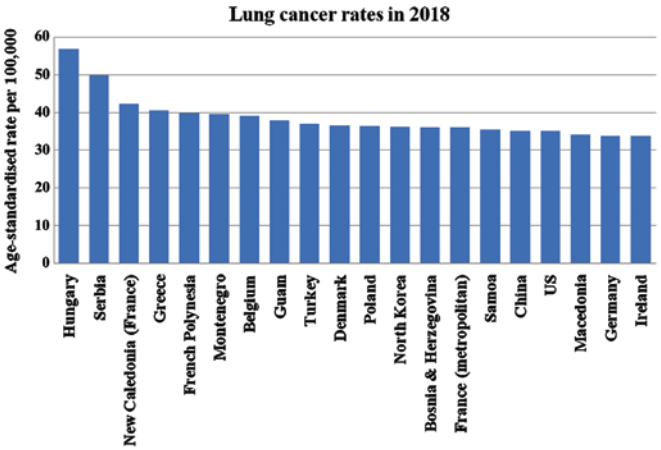

Figure 2. Top 20 countries affected by lung cancer (1).

Methodology. The adopted methodologies are presented in Fig. 3 and described as follows:

Data preprocessing: the microarray data were originally available as a CEL file, which is quantified and converted to gene expression values. After conversion into gene expression values, it is further quality checked and normalized to reduce variance among the data.

Differential expression analysis: the analysis of DEGs was performed using GEO2R tool available at NCBI-GEO. It is a user-friendly and interactive web-based tool that helps the researcher to compare groups of samples for the purpose of identifying DEGs across experimental conditions. We used adjusted P-value with Benjamini and Hochberg (19) false discovery rate and log fold-change as statistical metrics for evaluation purpose.

Results and Discussion

The considered datasets comprising 30 patients, out of which 18 samples belong to severe emphysematous tissue and 12 patients have normal/mildly emphysematous lung tissue from smokers suspicious of lung cancer. In order to understand the distribution of gene expression data among these two groups of samples, we depicted boxplot as shown in Fig. 4. It is observed from the boxplot (Fig. 4) that the values of gene expression lie between 0 and 300, while their 2nd quartile

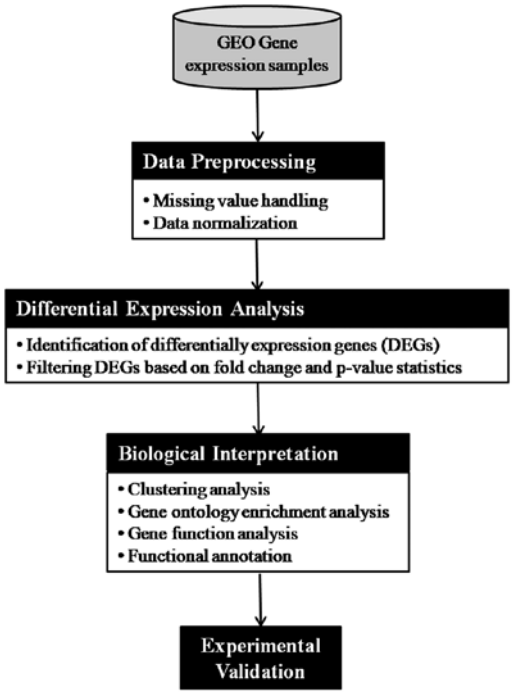

Figure 3. Flowchart of the gene expression analysis pipeline.

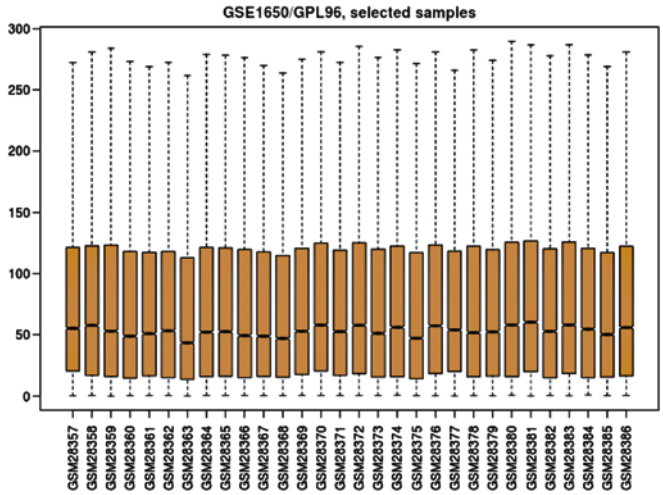

Figure 4. Boxplot of the 30 samples (18 samples from severe emphysematous tissue and 12 samples from normal/mildly emphysematous lung tissue from smokers suspicious of lung cancer).

(mean) fluctuates around 50. Thus, the gene expression data are uniformly distributed.

The Heatmap diagram shows the combined with clustering group genes and/or samples based on gene expression similarity pattern, which is helpful for the identification of commonly regulated genes, or gene signature associated with a disease. The heatmap diagram of our considered dataset is shown in Fig. 5, where rows represent genes and column represents samples. The changes of gene expression are depicted as color intensity; for instance, green color represents downregulated genes, red presents upregulated genes, and black represents no changes in the expression. It is observed from Fig. 5 that the majority of the genes are regulated, either down- or up-regulated.

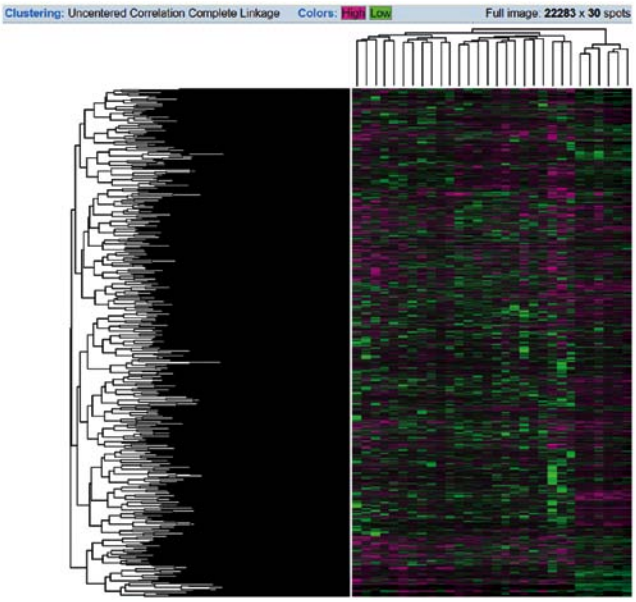

Figure 5. Heatmap diagram of the 30 samples. Rows represent genes, and columns represent samples. Red, upregulated genes; green, downregulated genes; black, unchanged gene expression.

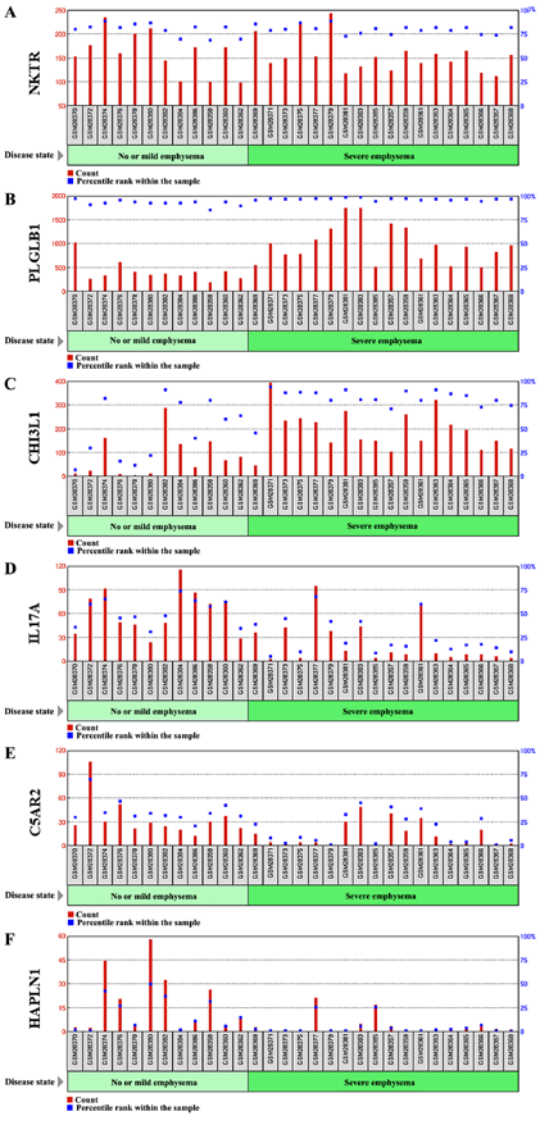

Figure 6. Gene expression profile graph of (A-F) six DEGs with a 4-fold change in the expression level.

Table I. List of differentially expressed genes along with their various scores.

Gene name	Adjusted P-value	P-value	t-statistics	B-statistics	logFC	FC	Gene description
NKTR	0.1544	0.0002	4.1685	0.4851	2.1583	4.4639	Natural killer cell triggering receptor
PLGLB1/ PLGLB2	0.2440	0.0019	3.4003	-1.2650	2.1000	4.2872	Plasminogen-like B1/B2
CHI3L1	0.1384	0.0002	-4.2923	0.7747	-2.0635	4.1801	Chitinase 3 like 1
IL17A	0.1264	0.0001	4.5501	1.3809	2.0311	4.0873	Interleukin 17A
C5AR2	0.1885	0.0005	3.8610	-0.2268	2.0277	4.0775	Complement component 5a receptor 2
HAPLN1	0.1953	0.0008	3.7205	-0.5476	2.0119	4.0332	Hyaluronan and proteoglycan link protein 1
LOXL1	0.1264	0.0001	-4.5643	1.4142	-1.9436	3.8466	Lysyl oxidase like 1
BRF1	0.1895	0.0007	3.7798	-0.4127	1.9285	3.8067	BRF1, RNA polymerase III transcription initiation factor 90 kDa subunit
CSF3	0.3087	0.0046	3.0529	-2.0141	1.9089	3.7552	Colony stimulating factor 3
PPM1A	0.1885	0.0004	3.9522	-0.0170	1.9064	3.7487	Protein phosphatase, Mg2+ /Mn2+ dependent 1A
UBR2	0.1351	0.0001	-4.3783	0.9764	-1.8816	3.6849	Ubiquitin protein ligase E3 component n-recognin 2
CLCA3P	0.2905	0.0031	3.2114	-1.6766	1.8733	3.6637	Chloride channel accessory 3, pseudogene
MYH7/ MYH6	0.1885	0.0006	3.8079	-0.3485	1.8698	3.6549	Myosin, heavy chain 7, cardiac muscle, beta/ myosin heavy chain 6
ZNF214	0.2418	0.0018	3.4102	-1.2431	1.8664	3.6462	Zinc finger protein 214
PCSK1	0.1544	0.0002	4.2011	0.5612	1.8579	3.6248	Proprotein convertase subtilisin/kexin type 1
PTGER3	0.1885	0.0006	3.8053	-0.3544	1.8309	3.5575	Prostaglandin E receptor 3
GYS2	0.2458	0.0020	3.3823	-1.3045	1.8290	3.5530	Glycogen synthase 2
BCAN	0.2188	0.0014	3.5128	-1.0156	1.8263	3.5462	Brevican
KIF23	0.2012	0.0010	3.6177	-0.7803	1.8148	3.5181	Kinesin family member 23
HTR2B	0.1923	0.0007	-3.7608	-0.4560	-1.7822	3.4396	5-hydroxytryptamine receptor 2B
BMP2K	0.343	0.0087	2.8034	-2.5282	1.7688	3.4077	BMP2 inducible kinase
CHIT1	0.1544	0.0002	-4.1743	0.4987	-1.7674	3.4044	Chitinase 1
TNIP3	0.1885	0.0006	3.8506	-0.2507	1.7650	3.3988	TNFAIP3 interacting protein 3
PHLPP1	0.3203	0.0056	2.9788	-2.1690	1.7485	3.3601	PH domain and leucine rich repeat protein phosphatase 1
POSTN	0.2692	0.0026	3.2736	-1.5422	1.7481	3.3592	Periostin
CCL20	0.3597	0.0126	2.6482	-2.8359	1.7446	3.3511	C-C motif chemokine ligand 20
SMARCA2	0.3332	0.0074	2.8663	-2.4007	1.7365	3.3323	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
TSC22D2	0.3475	0.0111	2.7019	-2.7306	1.7225	3.3001	TSC22 domain family member 2
FOSB	0.4467	0.0368	2.1818	-3.6944	1.7159	3.2850	FosB proto-oncogene, AP-1 transcription factor subunit
GGA2	0.262	0.0024	3.3140	-1.4542	1.7134	3.2793	Golgi associated, gamma adaptin ear containing, ARF binding protein 2

Table I. Continued.

Gene name	Adjusted P-value	P-value	t-statistics	B-statistics	logFC	FC	Gene description
KYNU	0.3263	0.0067	2.9063	-2.3189	1.7128	3.2779	Kynureninase
CCDC88C	0.3197	0.0053	3.0028	-2.1190	1.7090	3.2693	Coiled-coil domain containing 88C
MCF2	0.343	0.0087	2.8022	-2.5305	1.7089	3.2692	MCF.2 cell line derived transforming sequence
PAICS	0.3197	0.0054	2.9931	-2.1393	1.7001	3.2491	Phosphoribosylaminoimidazole carboxylase; phosphoribosylaminoimidazolesuccinocarboxamide synthase
CREBZF	0.3047	0.0045	3.0666	-1.9851	1.6783	3.2004	CREB/ATF bZIP transcription factor
AFF2	0.2458	0.0019	3.3904	-1.2868	1.6748	3.1927	AF4/FMR2 family member 2
XIST	0.3861	0.0182	-2.4926	-3.1341	-1.6726	3.1880	X inactive specific transcript (non-protein coding)
BPESC1	0.3735	0.0153	2.5674	-2.9922	1.6687	3.1793	Blepharophimosis, epicanthus inversus and ptosis, candidate 1 (non-protein coding)
KRT17/JUP	0.2473	0.0021	-3.3627	-1.3476	-1.6661	3.1736	Keratin 17/junction plakoglobin
CALCRL	0.2458	0.0020	3.3835	-1.3019	1.6656	3.1724	Calcitonin receptor like receptor
SALL1	0.3352	0.0079	2.8388	-2.4566	1.6603	3.1609	Spalt like transcription factor 1
GRIK2	0.3457	0.0100	2.7456	-2.6440	1.6533	3.1455	Glutamate ionotropic receptor kainate type subunit 2
KLK13	0.2347	0.0017	3.4334	-1.1918	1.6488	3.1358	Kallikrein-related peptidase 13
NOL4	0.1885	0.0005	-3.9187	-0.0943	-1.6357	3.1073	Nucleolar protein 4
KCNV1	0.2012	0.0010	3.6356	-0.7400	1.6335	3.1027	Potassium voltage-gated channel modifier subfamily V member 1
GTSE1	0.2692	0.0025	3.2897	-1.5071	1.6184	3.0703	G2 and S-phase expressed 1
SPON1	0.1885	0.0004	-3.9605	0.0023	-1.6153	3.0637	Spondin 1
CST1	0.1264	0.0001	-4.6287	1.5662	-1.6132	3.0593	Cystatin SN
TSPAN2	0.456	0.0406	2.1368	-3.7713	1.6114	3.0556	Tetraspanin 2
PLD1	0.2628	0.0024	3.3081	-1.4670	1.6076	3.0473	Phospholipase D1
CDHR5	0.2692	0.0026	3.2810	-1.5259	1.5982	3.0276	Cadherin related family member 5
SULF1	0.2188	0.0014	-3.5003	-1.0433	-1.5974	3.0260	Sulfatase 1
SLC12A4	0.2922	0.0035	3.1634	-1.7797	1.5933	3.0175	Solute carrier family 12 member 4

The profile graph of the six DEGs having a 4-fold change in the expression, i.e., NKTR, PLGLB1, CHI3L1, IL17A, C5AR2, and HAPLN1 are depicted in Fig. 6.

Differential expression analysis. We performed the differential expression analysis (DEGs) between the two samples, i.e., between severe emphysematous lung tissue and normal/mildly emphysematous from smokers suspicious of lung cancer. We filtered DEGs with a significance level of 5% (P-value < =0.05) and had fold-change (FC) ≥2. In this way,

Table II. GO enrichment analysis of six differentially expressed genes.

Gene name	GO molecular function	GO biological process	Cellular component	PMID
NKTR	Cyclosporin A binding, peptidyl-prolyl cis-trans isomerase activity, unfolded protein binding	Protein peptidyl-prolyl isomerization, protein refolding	Cytosol, mitochondrion, nucleoplasm	20676357, 20676357, 21873635
PLGLB1/ PLGLB2	--	--	Extracellular region	UniProt
CHI3L1	Carbohydrate binding, chitin binding, extracellular matrix structural constituent	Apoptotic process, carbohydrate metabolic process, cartilage development, cellular response to tumor necrosis factor, lung development	Endoplasmic reticulum	12775711, 9492324, 8245017, 18403759, 16234240
IL17A	Cytokine activity	Apoptotic process, cell-cell signalling, cell death, cytokine-mediated signalling pathway, immune response, inflammatory response	Extracellular region, extracellular space	7499828, 8390535
C5AR2	Complement component C5a receptor activity, G protein-coupled receptor activity	Chemotaxis, complement receptor mediated signaling pathway, inflammatory response, negative regulation of tumor necrosis factor production	Basal plasma membrane, plasma membrane	21873635, 16204243, 22960554
HAPLN1	Extracellular matrix structural constituent conferring compression resistance, hyaluronic acid binding	Cell adhesion, central nervous system development, extracellular matrix organization, skeletal system development	Collagen-containing extracellular matrix, extracellular matrix	20551380, 21873635, 23979707

we obtained 623 DEGs which had FC ≥2 in the expression level between the two samples. Out of 623 DEGs, 6 genes have a 4-fold change in the expression level, while 47 DEGs have a 3-fold change in their expression level (Fig. 6). The list of DEGS show 3- and 4-fold change in the expression level, along with other statistics such as adjusted P-value, P-value, moderated t-statistics, B-statistics, log FC and FC (Table I).

We further performed the Gene Ontology (GO) functional enrichment analysis of six DEGs found to have a 4-fold change in their expression (Table II). From our DEGs analysis, it can be inferred that the NKTR gene was upregulated 4-fold. This gene is expressed in natural killer cells as a multi-domain structure (20) with a peptidyl-prolyl cis-trans isomerase activity in oligopeptides assisting protein folding (21) and a putative tumor-recognition complex participating in NK cells function (20). PLGLB1 is a 4-fold upregulated gene expressed a plasminogen-like protein B found to bind to lysine binding sites present in the kringle structures of plasminogen (22). Similarly, CHI3L1 expression by approximately 4-fold plays an important role in tissue remodeling, and helps to cope with the changes in environment, T-helper cell type 2 inflammatory response and interleukin-3 induced inflammation, as well as inflammatory cell apoptosis (23,24).

In conclusion, COPD is a lung disease ranked third as a reason for mortality worldwide (13) This disease is influenced by both genetic and environmental factors. Cigarette smokers are the topmost risk factor in the western world. COPD constitutes the leading cause of mortality related to environmental factors such as smoking. Exacerbation of COPD exhibits various symptoms that include cough, production of sputum or shortness of breath. It can be caused either by bacterial or viral infections or inhaled particles. The genetic factor can also be helpful in determining the frequency of this disease. In this study, we performed differential gene expression analysis of 30 samples belonging to two different tissue types - severe emphysematous tissue and normal/mildly emphysematous lung tissue from smokers suspicious of lung cancer. We identified approximately 623 DEGs having 2- or more fold-change in their expression level, out of which 6 genes have 4-fold change, and 47 genes have a 3-fold change in the expression. We also performed GO enrichment analysis which uncovers fruitful knowledge that can be further validated from wet lab.

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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.

Authors' contributions

GJ conceived and designed the study. MB provided study materials or patients and was responsible for the collection and assembly of data, data analysis and interpretation. Both authors were involved in writing the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics review submission for approval is not required for this work. There is no identifiable more than minimal risk for the following reasons: i) This study does not contain human participants or animals procedures performed by any of the authors; ii) the data were taken from publicly available resource (GEO Datasets) where data referable to patients were properly anonymized by submitters and informed consent was obtained by the investigators during the original data collection; and iii) any active dissemination, in addition to the intention to submit findings for publication is purely an academic discussion of the study topic, i.e., method vis-à-vis analysis of gene expression.

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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