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Identification of lung cancer oncogenes based on the mRNA expression and single nucleotide polymorphism profile data

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This study aimed to identify the oncogenes associated with lung cancer based on the mRNA and single nucleotide polymorphism (SNP) profile data. The mRNA expression profile data of GSE43458 (80 cancer and 30 normal samples) and SNP profile data of GSE33355 (61 pairs of lung cancer samples and control samples) were downloaded from Gene Expression Omnibus database. Common genes between the mRNA profile and SNP profile were identified as the lung cancer oncogenes. Risk subpathways of the selected oncogenes with the SNP locus were analyzed using the iSubpathwayMiner package in R. Moreover, protein-protein interaction (PPI) network of the oncogenes was constructed using the HPRD database and then visualized using the Cytoscape. Totally, 3004 DEGs (1105 up-regulated and 1899 down-regulated) and 125 significant SNPs closely related to 174 genes in the lung cancer samples were identified. Also, 39 common genes, like PFKP (phosphofructokinase, platelet) and DGKH-rs11616202 (diacylglycerol kinase, eta) that enriched in sub-pathways such as galactose metabolism, fructose and mannose metabolism, and pentose phosphate pathway, were identified as the lung cancer oncogenes. Besides, PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1), RORA (RAR-related orphan receptor A), MAGI3 (membrane associated guanylate kinase, WW and PDZ domain containing 3), PTPRM (protein tyrosine phosphatase, receptor type, M), and BMP6 (bone morphogenetic protein 6) were the hub genes in PPI network. Our study suggested that PFKP and DGKH that enriched in galactose metabolism, fructose and mannose metabolism pathway, as well as PIK3R1, RORA, and MAGI3, may be the lung cancer oncogenes.

Key words: lung cancer, single nucleotide polymorphism (SNP), function analysis, differentially expressed gene, oncogenes

Lung cancer is one of the most common malignancies with an increasing morbidity and mortality and is a worldwide leading cause of cancer-related death with a 5-year survival rate from 13% to 15% [1]. Mechanism of lung cancer is complicate, mainly due to the late diagnosis and lack of effective treatment [2]. Therefore, underlying the molecular profiles of lung cancer as well as elucidating the roles of oncogenes and tumor suppressors in the development of this malignancy is expected to identify the molecular targets for lung cancer prediction and treatment.

Previous studies have demonstrated that environmental factors like smoking and air pollution, and gene polymorphisms were the main factors contributing to the lung cancer progression and metastasis [3]. Chemical constituents in tobacco smoke including compounds of the carcinogenic polycyclic type were the carcinogen for lung cancer [4]. A previous study reveals that mutations of the tumor suppressor genes are a main reason for lung cancer progression, such as the prevalence of p53 mutational patterns G to T transversions is 30% and p53 mutations in lung cancer can be attributed to the direct DNA damage [5]. Besides, increasing evidences have demonstrated that genes with single nucleotide polymorphism (SNP) played crucial roles in many cancers [6, 7]. For instance, rs1051730 and rs8034191 that map to the region of 15q25.1 containing PSMA4 (proteasome alpha 4 subunit isoform 1) and the nicotinic acetylcholine receptor gene CHRNA3 and CHRNA5 (nicotinic acetylcholine receptor alpha subunits 3 and 5), are related to the lung cancer risk [8]. Also, variations of TERT(rs2736100)-CLPTM1L(rs4975616) and CHRNA5-

Abbreviations: SNP – single nucleotide polymorphism; PPI – protein-protein interaction; DEGs – differentially expressed genes

CHRNA3 (rs8042374) influence the risk of lung cancer in non-smokers [9].

Lai *et al.* [10] using the GSE33355 microarrays to study that SNP rs10248565 in HDAC9 was a novel genomic aberration biomarker of lung adenocarcinoma in non-smoking women. Chan *et al.* [11] used the GSE43458 microarrays to construct the molecular probes for lung cancer, while Kabbout *et al.* [12] proved the suppression role of ETS2 in human non-small lung cancer cells by inhibiting of the MET proto-oncogene using the same profile data. In this study, we used the microarray analysis to screen the differentially expressed genes (DEGs) in lung cancer based on the mRNA microarray GSE43458 and SNP microarray GSE33355. Comprehensive bioinformatics was used to construct the protein-protein interaction (PPI) network and to select the risk subpathways of the DEGs to investigate the functions and pathways of hub genes associated with lung cancer. Our study aims to select several key oncogenes in lung cancer and provide theoretical basis for the lung cancer treatment.

Materials and methods

Data resources and data preprocessing. The mRNA expression profile data of GSE43458 [12], including 80 lung cancer samples and 30 normal samples, was downloaded from the GEO (Gene Expression Omnibus) database in NCBI (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/geo/) based on the platform of GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]. The downloaded CEL files were normalized using the affy package in R [13], and 20254 probes were obtained after normalization.

In addition, the SNP microarray data of GSE33355 [14], containing 61 lung cancer samples and 61 normal samples from non-smoking females, was downloaded from the GEO database in NCBI based on the platform of GPL6801 [GenomeWideSNP_6] Affymetrix Genome-Wide Human SNP 6.0 Array. The downloaded SNP files were subtyped using the crlmm package [15], hapmapsnp package [16], and pd.genomewidesnp.6 package [17] in R language to eliminate the samples with subtyped success rate lower than 90%. Finally, 118 samples (60 lung cancer samples and 58 controls) with 847,576 probes were chosen in this study.

Screening of DEGs and significant SNPs. The DEGs in the lung cancer samples compared to the controls were screened using the limma package in R [18]. The t-test and Bonferroni test [19] were used to adjust the p-value of each gene. The adj.p-value < 0.05 and |log2 FC (fold change)| > 0.5 were chosen as the threshold. Additionally, plink software is a tool set for whole-genome association and population-based lingage analyses [20]. The assoc in plink [21] was used to select the significant SNPs in lung cancer samples compared with the controls with the p-vaule < 0.05.

Identification of lung cancer oncogenes. In order to select the oncogenes of lung cancer, the genes that both corresponding to the selected significant SNPs and the selected DEGs were analyzed as the oncogenes.

Genes associated with one or more than one SNP were screened. If there was more than one SNPs for one gene, the most significant p-value for one SNP was considered as the p-value for one gene. The common genes were screened that both in the selected DEGs set and the gene set corresponding to the SNPs. The common genes that fit into the χ^2 square distribution with the 2K freedom degree, were tested using the Fisher's combination method [22] based on the formula of $\chi^2 = -2\sum_{i=1}^{k} \ln P_i$. Wherase, K = 2, P_i stands for the p-value for one common gene that associated with the p-value of this DEG as well as the significant p-value for this gene with the significant SNP. The p-value < 0.05 was chosen as the threshold.

Risk subpathways selection of the oncogenes. The pathways of the selected oncogenes were analyzed using the iSubpathwayMiner package in R [23]. The p-value < 0.05 was chosen as the threshold.

Construction and analysis of the PPI network of the oncogenes. HPRD (Human Protein Reference Database) is a database of curated proteomic information pertaining to human proteins [24]. In order to investigate the interactions among the selected lung cancer oncogenes, PPI network of 39 oncogenes was constructed using the HPRD database and then visualized using the Cytoscape software [25]. Additionally, topological analysis of PPI network was conducted to calculate the node degree of each gene.

Results

Screening of DEGs and significant SNPs. Totally, 3004 DEGs (1105 up-regulated and 1899 down-regulated) in the lung cancer samples were screened. Also, 125 significant SNPs (that located in gene coding region, upstream and downstream control region) were identified to be associated with174 genes in the lung cancer samples.

Risk subpathways selection of the oncogenes. A total of 39 common genes between the selected DEGs and genes associated with the significant SNPs were identified as the lung cancer oncogenes (Table 1). The risk sub-pathways of the selected oncogenes were shown in Table 2. The PFKP- rs11251105 (phosphofructokinase, platelet) and DGKH-rs11616202 (diacylglycerol kinase, eta) were 2 oncogenes that enriched in sub-pathways such as galactose metabolism, fructose and mannose metabolism, and pentose phosphate pathway, implying their important roles in lung cancer.

PPI network of the oncogenes and topological analysis. The PPI network of the selected 39 lung cancer oncogenes was constructed, including 281 nodes and 268 gene interaction pairs (Figure 1). There were 27 oncogenes and 254 non-oncogenes that interacted with the selected oncogenes. The topological analysis of the PPI network showed that PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1), RORA (RAR-related orphan receptor A), MAGI3 (membrane associ-

Table 1. The selected 39 oncogenes of lung cancer

Gene	Limma p-value	dbsnpID	Chromosome	SNP p-value
ADRA2A	1.08E-06	rs17129502	10	0.04948
AGR3	0.003068	rs4329181	7	0.01242
AGTR2	0.000109	rs5950506	X	0.0474
BMP6	3.98E-16	rs267203	6	0.04659
C10orf84	4.02E-10	rs933018	10	0.04948
CACNA2D1	0.002882	rs37109	7	0.04548
CACNB4	0.000128	rs16830650	2	0.02636
CD180	2.84E-06	rs7705112	5	0.04955
CLDN18	4.65E-10	rs11706173	3	0.04548
CNTN4	2.81E-19	rs11714941	3	0.04766
CRBN	2.96E-13	rs13318984	3	0.04941
DGKH	8.68E-07	rs11616202	13	0.03196
FGF10	5.35E-15	rs1004720	5	0.04934
GRIP1	0.000165	rs17779811	12	0.02771
IL20RB	0.000398	rs16847364	3	0.01341
KLF4	1.41E-16	rs10119737	9	0.02925
LPHN3	2.25E-20	rs12510186	4	0.04955
LRIG1	0.000138	rs9818858	3	0.04955
MAGI3	3.14E-08	rs1624335	1	0.02501
MSRB3	3.52E-19	rs7970955	12	0.04355
MYH10	4.72E-16	rs6503137	17	0.02647
PCDH17	4.52E-13	rs7996198	13	0.03373
PCDH20	0.002186	rs17088665	13	0.04744
PCDH9	4.88E-05	rs17078274	13	0.0291
PFKP	5.00E-07	rs11251105	10	0.04927
PIK3R1	6.53E-10	rs7705112	5	0.04955
PIP5K1B	6.92E-15	rs1576586	9	0.04941
PTPRM	2.22E-15	rs16953828	18	0.02144
RGS6	0.0014	rs8011978	14	0.03963
RORA	4.52E-09	rs8039843	15	0.04365
RP1	3.85E-09	rs4737807	8	0.0417
S1PR1	1.19E-24	rs11164248	1	0.03042
SEMA6A	2.49E-20	rs684730	5	0.04744
SLC16A7	0.00427	rs3763979	12	0.04747
SLC1A1	3.81E-09	rs4376542	9	0.04175
SMARCA2	1.18E-06	rs942400	9	0.0474
SOX5	3.49E-15	rs7967757	12	0.01878
TNR	4.44E-12	rs10798415	1	0.04167
ZNF521	3.63E-07	rs1674614	18	0.04546

Limma p-value: the p-value of one oncogene analyzed in the mRNA expression profile; SNP p-value: the p-value of one oncogene analyzed in the SNP data.

ated guanylate kinase, WW and PDZ domain containing 3), AMARCA2, PTPRM (protein tyrosine phosphatase, receptor type, M), and BMP6 (bone morphogenetic protein 6) were the genes with top 5 node degree (Table 3).

Discussion

In this study, we used the mRNA expression profile and the SNP profile to select the oncogenes of lung cancer. PFKP-rs11251105 and DGKH-rs11616202 that enriched in sub-pathways such as galactose metabolism, fructose and mannose metabolism, and pentose phosphate pathway, were identified as the lung cancer oncogenes. Besides, PIK3R1-rs7705112, RORA-rs8039843, and MAGI3-rs1624335 were the hub genes in PPI network.

Our findings displayed that PFKP-rs11251105 and DGKH-rs11616202 were the two oncogenes enriched in the risk sub-pathways galactose metabolism, fructose and mannose metabolism, and pentose phosphate pathway, suggesting their important roles in lung cancer. PFKP (encoded by

Table 2. The risk sub-pathways of selected oncogenes

PathwayID	pathwayName	pvalue	Genes
path:00052_2	Galactose metabolism	0.006591	PFKP
path:00052_4	Galactose metabolism	0.006591	PFKP
path:00052_8	Galactose metabolism	0.006591	PFKP
path:00052_1	Galactose metabolism	0.008232	PFKP
path:00052_6	Galactose metabolism	0.019646	PFKP
path:00051_10	Fructose and mannose metabolism	0.022884	PFKP
path:00010_5	Glycolysis / Gluconeogenesis	0.029328	PFKP
path:00010_7	Glycolysis / Gluconeogenesis	0.030932	PFKP
path:00051_3	Fructose and mannose metabolism	0.030932	PFKP
path:00010_2	Glycolysis / Gluconeogenesis	0.032534	PFKP
path:00051_8	Fructose and mannose metabolism	0.032534	PFKP
path:00051_15	Fructose and mannose metabolism	0.032534	PFKP
path:00010_6	Glycolysis / Gluconeogenesis	0.034134	PFKP
path:00051_2	Fructose and mannose metabolism	0.034134	PFKP
path:00051_6	Fructose and mannose metabolism	0.035731	PFKP
path:00030_1	Pentose phosphate pathway	0.038917	PFKP
path:00030_2	Pentose phosphate pathway	0.038917	PFKP
path:00030_3	Pentose phosphate pathway	0.043676	PFKP
path:00561_5	Glycerolipid metabolism	0.059377	DGKH
path:00561_1	Glycerolipid metabolism	0.071757	DGKH
path:00561_2	Glycerolipid metabolism	0.079414	DGKH
path:00564_5	Glycerophospholipid metabolism	0.079414	DGKH
path:00564_9	Glycerophospholipid metabolism	0.079414	DGKH
path:00564_2	Glycerophospholipid metabolism	0.091538	DGKH
path:00564_3	Glycerophospholipid metabolism	0.096044	DGKH
path:00564_8	Glycerophospholipid metabolism	0.096044	DGKH
path:00564_1	Glycerophospholipid metabolism	0.099036	DGKH
path:00564_6	Glycerophospholipid metabolism	0.099036	DGKH
path:00564_7	Glycerophospholipid metabolism	0.099036	DGKH
path:00564_10	Glycerophospholipid metabolism	0.100529	DGKH

PFKP gene) is a platelet isoform of phosphofructokinase that catalyzes the irreversible conversion of fructose-6-phosphate to fructose-1,6-bisphosphate and is a key regulator enzyme in glycolysis [26], while DGKH (encoded by *DGKH* gene) is a member of the diacylglycerol kinase enzyme family of proteins that are involved in regulating the intracellular concentrations of diacylglycerol and phosphatidic acid [27]. Roles of DGKH and PFKP in lung cancer have not been fully discussed. However, Oparina *et al.* proved that expression stability of the glycolytic enzyme encoding genes affecting the malignant and mRNA expression level of PFKP in kidney cancer was higher than the normal samples [28]. Molatore and his colleagues proved that DGKH was up-regulated in rats with pheochromocytoma and may be a biomarker for clinical use [29]. Thus, PFKP and DGKH may be important in lung cancer development. Besides, previous study has referred that due to high consumption of dairy foods or reduced galactose metabolism, increasing exposure to galactose was related to the ovarian cancer development [30]. Also, Tachibana *et al.* proved the association between

Table 3. The selected oncogenes with top 5 node degree in PPI network

Gene	Node degree
MYH10	5
SEMA6A	5
KLF4	6
TNR	6
CACNB4	7
S1PR1	7
ADRA2A	8
AGTR2	8
GRIP1	8
BMP6	9
PTPRM	9
SMARCA2	11
MAGI3	12
RORA	15
PIK3R1	127

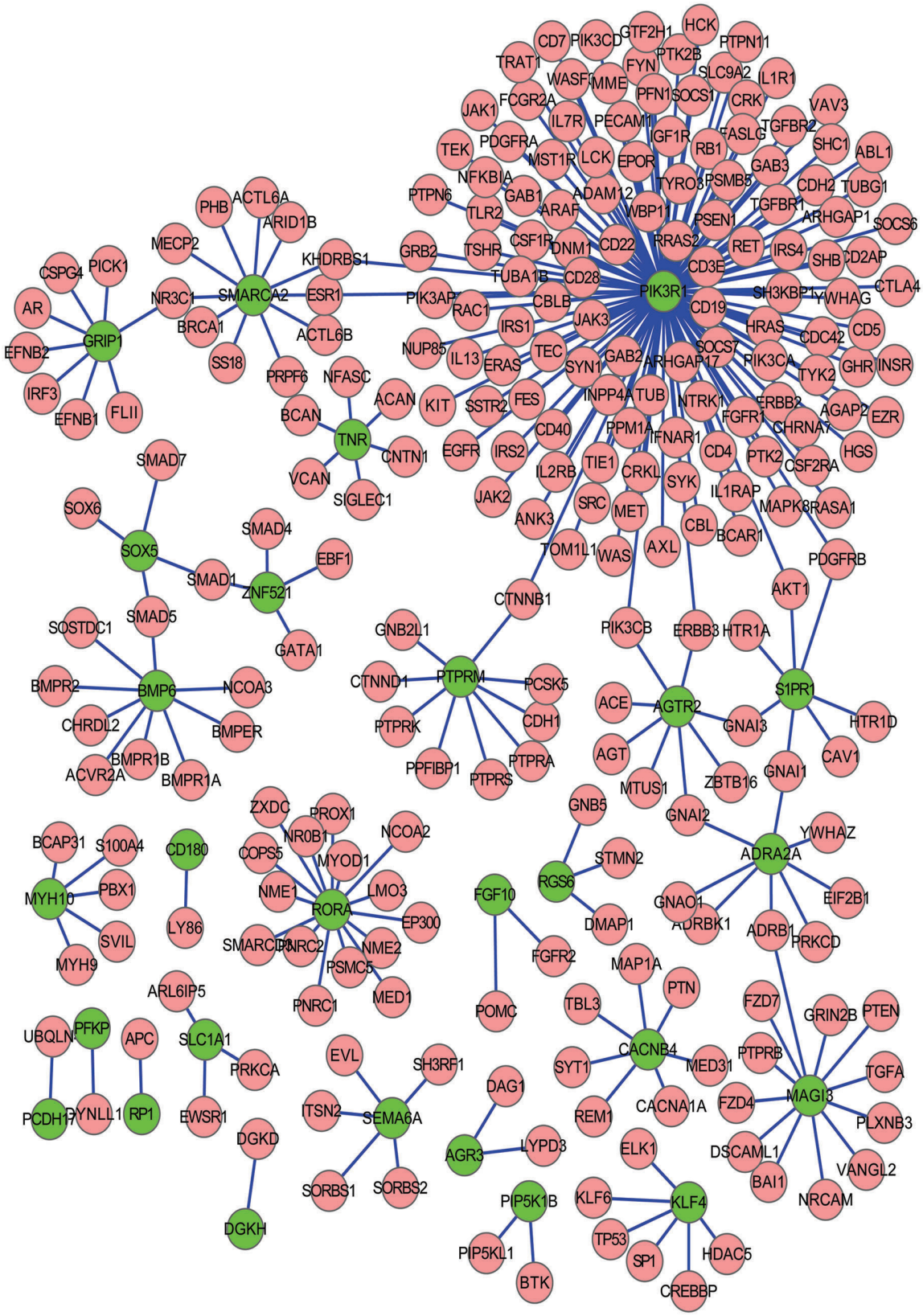

Figure 1. Protein-protein interaction network of the lung cancer oncogenes
Green nodes represent the lung cancer oncogenes and red nodes represent the non-oncogenes that interacted with the lung cancer oncogenes.

fructose and lung cancer [31]. Martin *et al.* said that the glycolysis pathways and the enzymes were up-regulated in non-small cell lung cancer cells [32]. Based on our study, we speculated that PFKP and DGKH maybe two oncogenes contributing to the lung cancer development via involving in the galactose metabolism, fructose and mannose metabolism.

PIK3R1 (encoded by *PIK3R1* gene), also known as GRB1, p85-ALPHA, is a regulatory subunit that plays an important role in the metabolic actions of insulin and is related to protein phosphatase binding [33]. Overexpression of PIK3R1 was involved in the SRSF2 RNA expression in H358 lung cancer [34]. Also, PIK3R1 that associated with the cell apoptosis, was overexpressed in non-small cell lung cancer [35]. Mutation of PIK3R1 has been observed in the thyroid cancer [36], ovarian and breast cancer [37], and the PIK3R1 3'untranslated region was the binding site for miR-127-3p including SNP-56 in bladder cancer [38]. Our data showed that down-regulated PIK3R1 with the SNP-rs7705112 was the hub protein in PPI network, suggesting its promoter role in lung cancer.

RORA (encoded by *RORA* gene) is a member of the NR1 subfamily of nuclear hormone receptors [39]. RORA binds to the promoter region of let-7α2 gene in lung cancer A549 cells [40]. Shi *et al.* said that silencing of RORA attenuated the cell apoptosis while overexpression of RORA enhanced the cell apoptosis in patients with pulmonary emphysema [41]. Also, role of RORA associated with T cell cytokines in the non-small cell lung cancer was also performed by Neurath [42]. Hence, RORA may be a contributor in lung cancer development. Our findings displayed that RORA was the hub protein in the PPI network, indicating that RORA may be an oncogene in lung cancer development.

Meanwhile, MAGI3 is a protein that function as a scaffolding protein at cell-cell junctions, thereby regulating various cellular and signaling processes [43]. It has been reported that MAGI3 could cooperate with PTEN to modulate the AKT1 activity [44, 45]. Tang *et al.* proved that the overexpression of AKT1 and loss expression of PTEN (phosphatase and tensin homolog) in non-small cell lung cancer resulted to a poor prognosis [46]. Therefore, MAGI3 may be important in lung cancer prognosis. Also, role of MAGI3 in lung cancer via the WNT/PCP signaling pathway has been reported from the study of Katoh *et al.* [47]. In this study, the MAGI3 was the hub protein in the PPI network, implying that MAGI3 may be an oncogene in lung cancer prognosis.

In conclusion, our study attempted to investigate several crucial genes with SNPs that associated with the mechanism of lung cancer. PFKP and DGKH that enriched in galactose metabolism, fructose and mannose metabolism pathway, and PIK3R1, RORA, and MAGI3, may be the lung cancer oncogenes. Our study may provide basis for the lung cancer mechanism research. However, further experimental studies on these selected oncogenes are still needed since our findings were based only on the microarray data downloaded from GEO database.

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