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Reveals new lung adenocarcinoma cancer genes based on gene expression

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Abstract. – BACKGROUND: Lung adenocarcinoma (LAC) is the most common type of lung cancer, accounting for 30-35% of all cases.

AIM: In this study we aim to predict potential genes and confirm pathways which are associated with LAC.

MATERIALS AND METHODS: By using the meta-analysis method, GSE10072 and GSE 2514 datasets were merged to find potential genes and pathways which are associated with LAC.

RESULTS: Our analysis indicated identified differentially expressed genes enriched in multicellular organismal metabolic process, gland development, and urogenital system development. Further, we predicted genes including EGF-like domain might be the potential target genes for further study, such as NGX6, MUC17, and Nel. In addition, a number of genes that associated with axon guidance, focal adhesion, and complement and coagulation cascades pathway might be also involved in LAC in a direct or indirectly manner.

CONCLUSIONS: Our analysis indicated identified differentially expressed genes enriched in multicellular organismal metabolic process, gland development, and urogenital system development We anticipate numerous advances in LAC research in the coming years based on our meta-analysis.

Key Words:
Lung adenocarcinoma, Meta-analysis, EGF-like domain, Pathway.

Introduction

Lung carcinomas are usually classified as small-cell lung carcinomas (SCLC) or non-small-cell lung carcinomas (NSCLC). NSCLC is histopathologically and clinically distinct from SCLC, and is further subcategorized as adenocarcinomas, squamous cell carcinomas, and large-cell carcinomas, of which adenocarcinomas are the most common[1].

Recently, many genetic factors have been proposed involved in lung adenocarcinoma (LAC), including several tumour suppressor genes (TP53, CDKN2A and STK11, NF1, ATM, RB1, APC, etc) along with tyrosine kinase genes (KRAS, EGFR, ERBB, EPHA3, NRAS, KDR, FGFR4 and NTRK etc) that may function as proto-onco-genes[2,3]. However, the mechanism of LAC is unclear and more potential genes are still necessary to mine. The development of microarray methods for large-scale analysis of gene expression[4] makes it possible to search systematically for molecular markers of cancer classification and outcome prediction in a variety of tumor types[5,6]. DNA microarray analysis as a global approach is applied to investigate whole genomic expression profiling and physiological mechanisms in health and disease[7,8]. Therefore, a high-throughput microarray experiment was also designed to analyze genetic expression patterns and identify potential genes to target for LAC[9]. These identified differentially expressed genes may play pivotal roles in lung tumorigenesis and may potentially serve as biomarkers in both diagnosis and prognosis of human lung cancer[10]. While meta-analysis provides a powerful tool for analyzing microarray experiments by combining data from multiple studies, it presents unique computational challenges. The Bioconductor package RankProd provides a new and intuitive tool for this purpose in detecting differentially expressed genes under two experimental conditions[11].

Here we measured transcription profiles of LAC by meta-analysis method and Gene Ontology (GO), domain enrichment analysis were used to predict potential genes and confirm pathways which are associated with LAC. These underlying genes and pathways are potential targets for future studies of LAC.

Materials and Methods

Microarray data (GSE2514 and GSE10072) have been deposited in Gene Expression Om-

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nibus. For the GSE2514 and GSE10072 datasets, the limma method[12] was used to identify differentially expressed genes (DEGs). The original expression datasets from all conditions were processed into expression estimates using the RMA method with the default settings implemented in Bioconductor, and then construct the linear model. The DEGs only with the fold change larger than 2 and *p*-value less than 0.05 were selected.

The Rank Product (RP) package[11] was used to identify the DEGs between controls and treatment in each experiment. Briefly, genes were ranked based on up- or down-regulation by the treatment in each experiment. Then, for each gene a combined probability was calculated as a rank product (RP). The RP values were used to rank the genes based on how likely it was to observe them by chance at that particular position on the list of DEGs. The RP can be interpreted as a *p*-value. To determine significance levels, the RP method uses a permutation-based estimation procedure to transform the p-value into an e-value that addresses the multiple testing problems derived from testing many genes simultaneously. Genes with a percentage of false-positives (PFP) ≤ 0.05 were considered differentially expressed between treatments and control in each experiment.

GO Enrichment and IntroPro Domain Analysis

Gene Ontology (GO) data and functional domain data were extracted using the DAVID[13]. GO terms and domains with less than 2 genes were discarded. Over-represented groups of GO terms and functional domains[14] were identified using a hypergeometric test, with a threshold of *p*-value < 0.01.

Pathway Analysis

We adopted an impact analysis that includes the statistical significance of the set of pathway genes but also considers other crucial factors such as the magnitude of each gene's expression change, the topology of the signaling pathway, their interactions, etc[15].

In this model, the Impact Factor (IF) of a pathway Pi is calculated as the sum of two terms:

$$IF\,(\mathrm{Pi}) = \log\left(\frac{1}{\mathrm{pi}}\right) + \frac{\sum_{g\in \mathrm{Pi}} |\mathrm{PF}|\,(g)}{|\Delta \mathrm{E}| \cdot N_{de}\,(\mathrm{Pi})}$$

The first term is a probabilistic term that captures the significance of the given pathway Pi from the perspective of the set of genes contained in it.

The second term is a functional term that depends on the identity of the specific genes that are differentially expressed as well as on the interactions described by the pathway (i.e., its topology).

Results

Effects of LAC on Transcript Levels in Human

After microarray analysis, the differentially expressed genes with the fold change larger than 2 and *p*-value less than 0.05 were selected. 340 genes from GSE2514 and 659 genes from GSE10072 were selected as DEGs. Finally, 262 common genes were selected for meta-analysis. Using the RankProd packages, 111 genes with a percentage of false-positives (PFP) < 0.05 were considered differentially expressed between treatments and control.

GO Enrichment Analysis

To gain insight into the biological processes associated with the regulated genes, we determined which GO terms were over-represented. In both treatments, significantly enriched GO terms ($p < 0.01$, hypergeometric test) were related with multicellular organismal metabolic process, gland development, and urogenital system development (Figure 1).

Domain Analysis

To add meaningful information to the results from the GO term enrichment, we extended our investigation to protein domains. Only functional domains common and significant (*p*-value < 0.01, hypergeometric test) within the LAC are shown (Table I). Most of significantly overrepresented groups included domains were related with EGF-like.

Pathway Enrichment Analysis

We adopted an impact analysis method that contained many factors including the statistical significance of the set of differentially expressed genes in the pathway, the magnitude of each gene's expression change, the topology of the signaling pathway, their interactions and so on. The impact analysis method yielded many significant pathways contained Axon guidance, Focal adhesion, and Complement and coagulation cascades and so on (Table II).

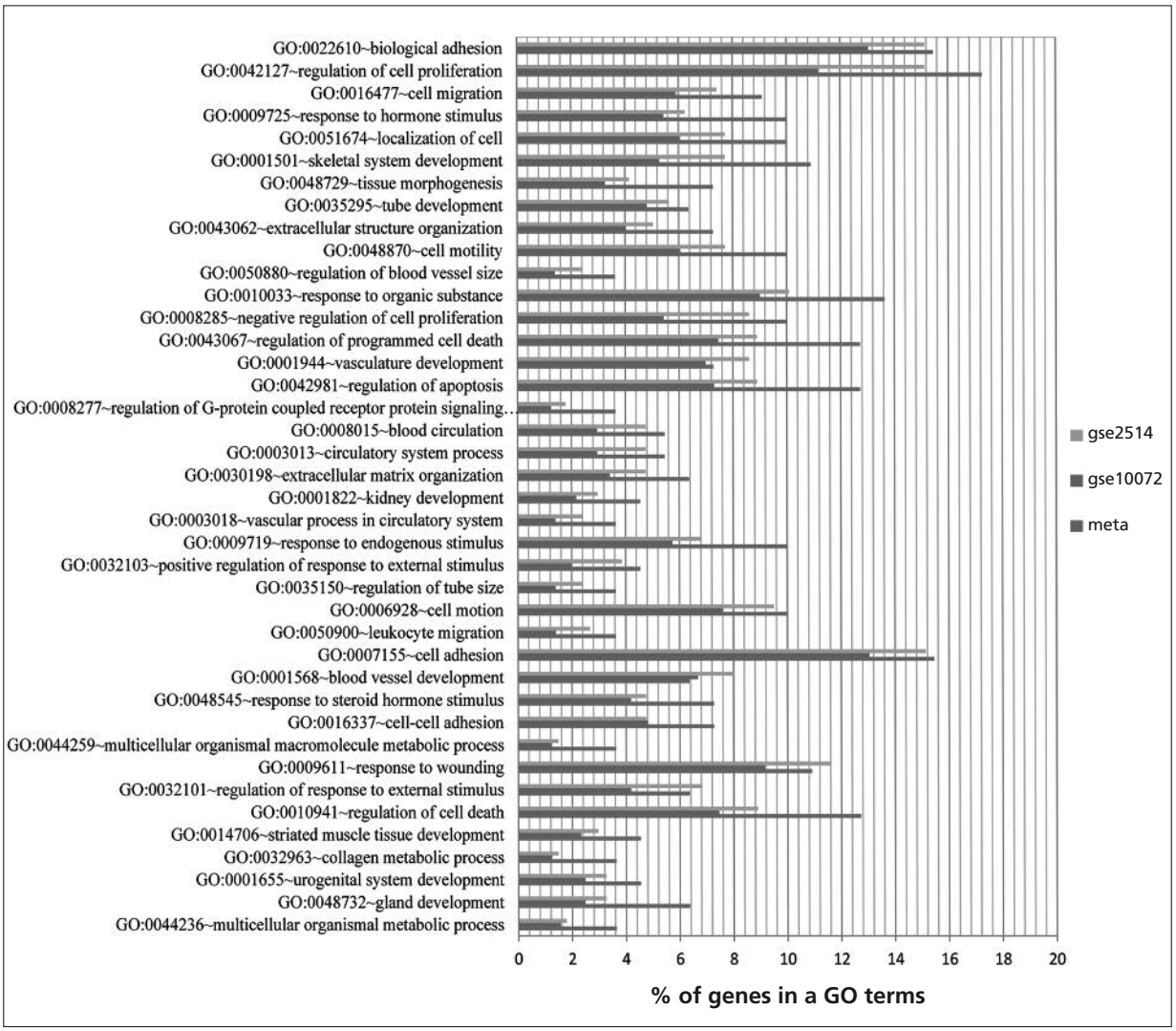

Figure 1. Differentially expressed genes within enriched GO terms in GSE10072, GSE2514 and a combination of both. Percentage of genes within each GO terms significantly regulated in each dataset: gse2514 (*green*), gse10072 (red) and a combination of both (*blue*). Only significantly enriched GO terms for all the treatments are shown (p-value < 0.01 using hypergeometric test).

Table I. Differentially expressed genes within enriched domains in LAC.

Category	Term	meta – *p*-value	gse10072 – *p*-value	gse2514 – *p*-value
IntrPro	IPR006210:EGF-like	0.001552	5.02E-11	6.70E-08
IntrPro	IPR000885:Fibrillar collagen, C-terminal	3.65E-05	2.45E-05	0.001033
IntrPro	IPR013032:EGF-like region, conserved site	0.009695	1.06E-11	4.90E-10
IntrPro	IPR006209:EGF	0.00797	8.22E-07	1.01E-06
IntrPro	IPR001007:von Willebrand factor, type C	0.001921	1.23E-07	9.77E-06
IntrPro	IPR008160:Collagen triple helix repeat	0.00182	2.31E-04	0.005557
IntrPro	IPR006985:Receptor activity modifying protein	1.13E-04	0.003893	0.00109
IntrPro	IPR000742:EGF-like, type 3	0.001295	3.86E-12	1.07E-09

Table II. Pathways involved in LAC.

Database name	Pathway name	Impact factor	*p*-value	Impact factor	*p*-value	Impact factor	*p*-value
		gse10072		gse2514		Meta	
KEGG	Axon guidance	8.736	0.001565	7.593	0.004331	10.264	3.93E-04
KEGG	Focal adhesion	2.33E+01	1.85E-09	1.91E+01	1.05E-07	18.103	2.62E-07
KEGG	Complement and coagulation cascades	1.82E+01	2.49E-07	2.89E+01	8.45E-12	6.93E+00	0.00779
KEGG	ECM-receptor interaction	2.45E+01	5.78E-10	1.74E+01	5.15E-07	20.453	2.81E-08
KEGG	TGF-beta signaling pathway	1.04E+01	3.49E-04	6.53E+00	0.011024	9.62E+00	7.06E-04
KEGG	Leukocyte transendothelial migration	6.16E+01	1.07E-25	1663.867	0	173.841	5.55E-74
KEGG	Cell adhesion molecules (CAMs)	4.86E+01	3.83E-20	9.59E+02	0	118.613	3.67E-50
KEGG	Pathways in cancer	1.06E+01	2.99E-04	7.02E+00	0.007194	5.86E+00	0.019593
KEGG	Bladder cancer	7.31E+00	0.005582	5.86E+00	0.019626	7.818	0.003549
KEGG	Tight junction	1.28E+01	3.90E-05	21.79	7.84E-09	4.78E+00	0.048609

Discussion

RP (Rank Product package) has proved to be a robust method for comparing microarray data from different sources and experiments[16]. Based on this strategy, our analysis has focused on the significantly differentially expressed genes to reveal transcriptional responses of each LAC sample. Our analysis indicated these genes enriched in multicellular organismal metabolic process, gland development, and urogenital system development. Of them, growth hormone receptor (GHR) is involved in multicellular organismal metabolic process. The interaction of surface GHR of A549 human lung epithelial cells with GH was observed using nanodiamond's unique spectroscopic signal via confocal Raman mapping[17]. In addition, the expression of estrogen receptor-α (ER-α) was observed mostly in cytoplasm of NSCL cell and the expression of progesterone receptor (PR) was observed mostly in nucleus. ER-α expression was significantly associated with female gender. The expression of PR was significantly associated with better clinicopathologic features[18].

The type 1 insulin-like growth factor receptor (IGF-1R), which benefits for gland development and is over-expressed or activated in human lung cancers, mediates cancer cell proliferation and metastasis. Silencing IGF-1R decreases the expression of invasive-related genes including matrix metalloproteinase-2 (MMP-2), MMP-9, and urokinase-plasminogen activator (u-PA), and the phosphorylation of protein kinase B (Akt) and ERK1/2 (exogenously-regulated-kinase ½). These results suggest that the silencing of IGF-1R has the potential to be an effective cancer gene therapy strategy for human lung cancer[19].

PAX-8 was shown previously to correlate with the mesenchyme to epithelial transition in urogenital system development, such as kidney development. PAX-8 was also found expressed at low levels in NSCL and the loss of PAX-8 is consistent with a mesenchymal phenotype for NSCL[20]. Further study found primary lung adenocarcinomas were negative for PAX8, whereas metastatic carcinomas from the kidney, ovary, endometrium, endocervix, thyroid and urinary tract were positive for PAX8[21].

Further, we identified several protein domains significant expressed in lung cancer. Most of significantly overrepresented groups were related with EGF (epidermal growth factor)-like domain. This result indicated genes containing EGF domain might be potential targets for further LAC study. The epidermal growth factor (EGF)-like domain is mainly involved in receptor-ligand interactions, extracellular matrix formation, cell adhesion and chemotaxis. Recently, a number of genes consisting of EGF domain have been demonstrated participating in LAC, such as ADAM28 (disintegrin and metalloproteinase domain-containing protein 28), ADAM12-L, Slit2 (Slit homologue 2 protein), and HYAL2. ADAM (a disintegrin and metalloproteinases) are a gene family that composed of several domains including epidermal growth factor (EGF)-like, propeptide, metalloproteinase, disintegrin, cysteine-rich, transmembrane and cytoplasmic tail domains. Study demonstrate that one of ADAM member,

ADAM28 is over-expressed and activated in human NSCL carcinomas, and suggest the possibility that ADAM28 plays a role in cell proliferation and progression of the human lung carcinoma[22]. ADAM12-L mRNA expression also has been suggested as an independent prognostic factor in resected p-stage I LAC, and is significantly correlated with tumor differentiation stage and postoperative cancer recurrence[23]. The Slit proteins are highly conserved in evolution and contain a putative signal peptide, four tandem arrays of leucine-rich repeats required for the Slit-Robo interaction and axon repulsion, seven to nine EGF repeats, an agrin-laminin-perlecan-Slit conserved spacer motif, and a cysteine knot. SLIT2 is frequently inactivated in NSCL carcinomas by promoter region hypermethylation and allele loss and is an excellent candidate for the lung tumor suppressor gene previously mapped to 4p15.2[24]. HYAL2 ((hyaluronoglucosaminidase 2) protein is comprised of an N-terminal signal peptide (amino acids 1-20) and an epidermal growth factor (EGF)-like domain at amino acids 365-469. Increased level of HYAL2 deletions in sputum of Stage I NSCL carcinomas patients was associated with pack-years of smoking HYAL2 mRNA expression was inversely correlated with lymphoma aggressiveness[25].

In addition, recent study demonstrated that some EGF-like domain genes are also associated with other cancer, although none study is present in LAC. Therefore, these genes could be the potential target to further study. For example, NGX6 (nasopharyugeal carcinoma associated gene 6) is a novel EGF-like domain-containing gene located at the high frequent loss of heterozygosity (LOH) region 9p21-22 associated with nasopharyngeal carcinoma (NPC). It is down-regulated in NPC and its over-expression can delay the cell cycle G0-G1 progression in NPC cells[26]. The primary structure of the MUC17 (mucin-17) protein harbours a signal peptide, a large tandemly repeated central domain (TR), two epidermal growth factor (EGF)-like domains, a SEA domain, a transmembrane domain (TM), and an 80 amino acid cytoplasmic tail. MUC17 expression was decreased in colon cancer[27]. The Nel (strongly expressed in neural tissues and containing epidermal growth factor (EGF)-like domains) gene, encodes a protein containing six EGF-like domains. Hypermethylation of the nel-like 1 gene is a common and early event and is associated with poor prognosis in early-stage esophageal adenocarcinoma[28].

We also noted top 3 significant pathways, namely, axon guidance, focal adhesion, and complement and coagulation cascades, which were high related to LAC. Recently, a number of axon guidance genes, such as Slit2, EphA2, SEMA3B (semaphoring-3B), SEMA5A and netrin-1 have been implicated in human lung cancers and could be promising targets for personalized anticancer therapies. Slit2 plays a vital role in axon guidance by signaling through Robo receptors. Overexpression of the receptor tyrosine kinase EphA2 could occur in the early pathogenesis of LAC. Knockdown of EphA2 decreased cell proliferation and migration[29]. SEMA3B, located at 3p21.3, is a secreted member of the semaphorin family important in axonal guidance. SEMA3B undergoes allele and expression loss in lung cancer and can function as a tumor suppressor that induces apoptosis in SEMA3B-inactivated tumor cells through the neuropilin-1 receptor by inactivating the Akt signaling pathway[30]. Identically, SEMA5A was found down-regulated expression, both at the transcriptional and translational levels, and associated with poor survival among nonsmoking women with NSCLC[31]. High levels of netrin-1 were found in NSCLC tumor samples. Interference with netrin-1 in human lung cancer cell lines was associated with UNC5H-mediated cell death *in vitro*[32].

There was evident that focal adhesion pathway associated with LAC progression. Paxillin is a 68 kDa focal adhesion protein, involved in growth factor receptor, integrin and oncogenic signaling such as v-src and BCR/ABL (break point cluster/Abelson). Paxillin was also localized to the focal adhesions in NSCL cancer and the possible role of paxillin in lung cancer cells was to decrease cell motility and aggressiveness as compared to normal controls[33]. Src activity was found increased in the anoikis-resistant human LAC cells when they were detached and cultured in suspension. The detachment-induced Src activation in the tumor cells compensates for the loss of cell survival signals caused by disruption of cell-matrix interactions and contributes to anoikis resistance of the tumor cells[34]. Suppression of contactin-1 expression abolished the ability of LAC cells to invade Matrigel *in vitro* as well as the polymerization of filamentous-actin and the formation of focal adhesion structures, and further resulted in extensive inhibition of tumor metastasis and in increased survival in an animal model[35]. In addition, focal adhesion kinase (FAK) and PTEN (phosphate and tensin ho-

molog) are also identified up-regulated expression in NSCLCs, and suggest their potential involvement in lung cancer progression[36-38].

Recent reviews indicate that complement and coagulation cascades pathway involved in LAC. Complement C3 precursor was elevated in LAC[39]. The C9 protein level was also shown 6.4-fold higher in squamous cell lung cancer patients[40]. Study suggested gC1qR (cell-surface receptor for complement component C1q) is an essential component of lamellipodia in human lung carcinoma A549 cells. Moreover, the gC1qR-depleted cells exhibited a reduced proliferation rate in culture as well as diminished tumorigenic and metastatic activities in grafted mice[41]. Malignant cells are often resistant to complement activation through the enhanced expression of complement inhibitors. NSCLC cells could produce soluble complement inhibitor factor H(FH), complement inhibitors factor I (FI), and C4b-binding protein (C4BP) to degrade the activated complement components and improve tumor development[42,43].

Conclusions

In conclusion, we have used meta-analysis method to analyze the whole genome transcription profile of LAC. Our analysis indicated identified differentially expressed genes enriched in multicellular organismal metabolic process, gland development, and urogenital system development. Further, our results predicted genes including EGF-like domain might be the potential target genes for further study, such as NGX6, MUC17, and Nel. In addition, a number of genes that associated with axon guidance, focal adhesion, and complement and coagulation cascades pathway might be also involved in LAC in direct or indirectly manner. We anticipate numerous advances in LAC research in the coming years based on our meta-analysis.

Lin Zhi-Feng and Shen Xiao-Yong are co-first Authors.

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