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Protein-protein interaction network analysis of children atopic asthma

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Abstract. – BACKGROUND, Asthma prevalence has increased very considerably in recent decades such that it is now one of the commonest chronic disorders in the world. In this present study, We constructed a protein-protein interaction (PPI) network by mapped the differentially expressed genes (DEGs) to the PPI data and performed Gene Ontology (GO) enrichment analysis of the PPI network. We aimed to explore the pathogenesis of atopic asthma by bioinformatics methods.

MATERIALS AND METHODS, To explore the pathogenesis of atopic asthma by bioinformatics methods, we obtained the global gene expression profile of pediatric asthmatic epithelium GSE18965 from Gene Expression Omnibus (GEO), and identified the differentially expressed genes between healthy nonatopic samples and atopic asthmatic samples

RESULTS, Total 12 DEGs were selected. Furthermore, we constructed a protein-protein interaction network by mapped the DEGs to the PPI data and performed Gene Ontology enrichment analysis of the PPI network. Total 15 GO terms were enriched and the enriched terms can be generally classified into two groups: cell cycle and immunity.

CONCLUSIONS, Our results confirmed the role of cell proliferation and immune system in the pathogenesis of atopic asthma. Besides, our PPI network is useful in investigating the complex interacting mechanisms of transcription factors and their regulated genes in atopic asthma.

Keywords:
Protein-Protein Interaction (PPI) network, DEG, Atopic asthmatic.

Introduction

Asthma is a chronic disease characterized by reversible airflow obstruction and airway inflammation that affect many people[1]. Asthma prevalence has increased very considerably in recent decades such that it is now one of the commonest chronic disorders in the world. According to the World Health Organization's statistics, an estimated 300 million people worldwide suffer from asthma, with 250,000 annual deaths attributed to the disease[2]. Asthma is manifesting as two major phenotypes, nonatopic (intrinsic) asthma, and atopic (extrinsic) asthma[3], which is the focus of this present study. Atopic asthma is the dominant form of the disease throughout the school years and into young adulthood[4,5]. For most patients, asthma persistence is associated with recognizable risk factors including atopic disease, recurrent wheezing, and a parental history of allergy and/or asthma[6]. Evidence indicates that therapy with current anti-inflammatory medications does not alter the natural history and prevent progression of the underlying disease[7-10].

Asthma is thought to be caused by a combination of genetic and environmental factors[11]. Several environmental risk factors have been associated with asthma development and morbidity in children. Recent studies show a relationship between exposure to air pollutants (e.g. from traffic) and childhood asthma[12].Other possible allergens that may trigger allergic asthma include: dust mites, pollen, mould animal dander and so on. Upon exposure to the allergen, the airways become constricted and inflamed which affects breathing. Several candidate genes were implicated in the development of atopy and asthma to date, such as glutathione S-transferase mu 1 (GSTM1)[13,14], signal transducer and activator of transcription 6 (STAT6)[15], interleukin 5 (IL5)[16], IL10[17], IL13[18] and so on. Most of these genes are related to the immune system or to modulating inflammation.

In this present study, we investigated the global gene expression profile of pediatric asthmatic epithelium, and identified the differentially ex-

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pressed genes between healthy nonatopic samples and atopic asthmatic samples. We constructed a protein-protein interaction (PPI) network by mapped the DEGs to the PPI data and performed Gene Ontology enrichment analysis of the PPI network. We aimed to explore the pathogenesis of atopic asthma by bioinformatics methods.

Materials and Methods

Affymetrix Microarray Data

The transcription profile of GSE18965 was obtained from GEO database of National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/) which is based on the Affymetrix Human Genome U133A Array. Children (2.4-16.9 yr) with and without mild asthma were recruited before elective surgery. Children with asthma had not taken inhaled/oral glucocorticosteroids for at least 1 month before surgery. Epithelial cells were collected by bronchial brushing and cultured [19]. Total 16 chips including 9 samples of healthy nonatopic and 7 samples of atopic asthmatic were used for analysis.

Protein-Protein Interaction (PPI) Data

The Human Protein Reference Database (HPRD)[20] is a protein database accessible through the internet. The Biological General Repository for Interaction Datasets (BioGRID)[21] is a curated biological database of protein-protein and genetic interactions. Total 39240 pairs of protein protein interactions (PPI) were collected from HPRD and 379426 pairs of PPI were collected from BioGRID. Combined the two databases, total 326119 unique PPI pairs were collected.

Differentially Expressed Genes (DEGs) Analysis

For the GSE18965 dataset, the limma method[22] was used to identify differentially expressed genes(DEGs). The original expression datasets from all conditions were extracted into expression estimates, and then constructed the linear model. The DEGs only with the fold change value larger than 1.4 and *p*-value less than 0.05 were selected.

PPI Network Construction

For demonstrating the potential PPI relationship, we used the PPI data that have been collected from HPRD and BioGRID databases to match the interactions between two DEGs. Based on the above datasets, PPI network was constructed using the Cytoscape[23].

Gene Ontology Enrichment Analysis

Gene Ontology (GO) analysis has become a commonly used approach for functional studies of large-scale genomic or transcriptomic data[24].

DAVID[25], a high-throughput and integrated data-mining environment, analyzes gene lists derived from high-throughput genomic experiments. We used the DAVID to identify over-represented GO categories in biological process based on the hypergeometric distribution with the count larger than 2 and the False Discovery Rate (FDR) less than 0.05.

Results

Microarray Data Analysis

To get the differentially expressed genes between healthy nonatopic samples and atopic asthmatic samples, the publicly available microarray dataset GSE18965 was obtained from GEO. After microarray analysis, the differentially expressed genes with the fold change value larger than 1.4 of GSE18965 and p-value less than 0.05 were selected as DEGs. Total 12 DEGs were selected, including SLC16A3, POLH, TPP1, MYO6, HSP90B1, ACO1, ACSL1, ANKRD12, TOP1, SLC2A14, FFAR2 and DAB2.

Protein-Protein Interaction Network Construction in Atopic Asthma

To construct PPI network, we obtained the PPI interaction data from two databases, HPRD and BioGRID. Total 326119 unique PPI pairs were used to construct a protein-protein interaction (PPI) network by integrating above PPI databases in human (Figure 1). In this network, the proteins TOP1, TPP1, ACO1, POLH and MYO6 with high degrees form a local network which suggesting these proteins may play an important role in the development of atopic asthmatic.

GO Enrichment Analysis of PPI Network

To investigate the function changes in the development of atopic asthmatic, we used the DAVID to identify over-represented GO categories in biological process. The *p*-value less than 0.05 and count larger than 2 were chose as threshold. Total 15 GO terms were enriched among these genes in PPI network (Table I) and most of

Table I. The enriched GO terms in PPI network.

Term	Des	*p*-value	FDR
GO:0022403	cell cycle phase	0.003909	5.299184
GO:0006259	DNA metabolic process	0.006857	9.121837
GO:0022402	cell cycle process	0.009301	12.1826
GO:0007049	cell cycle	0.021938	26.53587
GO:0051301	cell division	0.02289	27.524
GO:0000279	M phase	0.028055	32.6714
GO:0000278	mitotic cell cycle	0.034855	38.93294
GO:0006974	response to DNA damage stimulus	0.035376	39.3898
GO:0002449	lymphocyte mediated immunity	0.055489	54.77989
GO:0045786	negative regulation of cell cycle	0.06395	60.09691
GO:0002443	leukocyte mediated immunity	0.067772	62.30372
GO:0000075	cell cycle checkpoint	0.071581	64.38923
GO:0033554	cellular response to stress	0.07485	66.09344
GO:0016192	vesicle-mediated transport	0.077179	67.2606
GO:0010564	regulation of cell cycle process	0.088919	72.59916

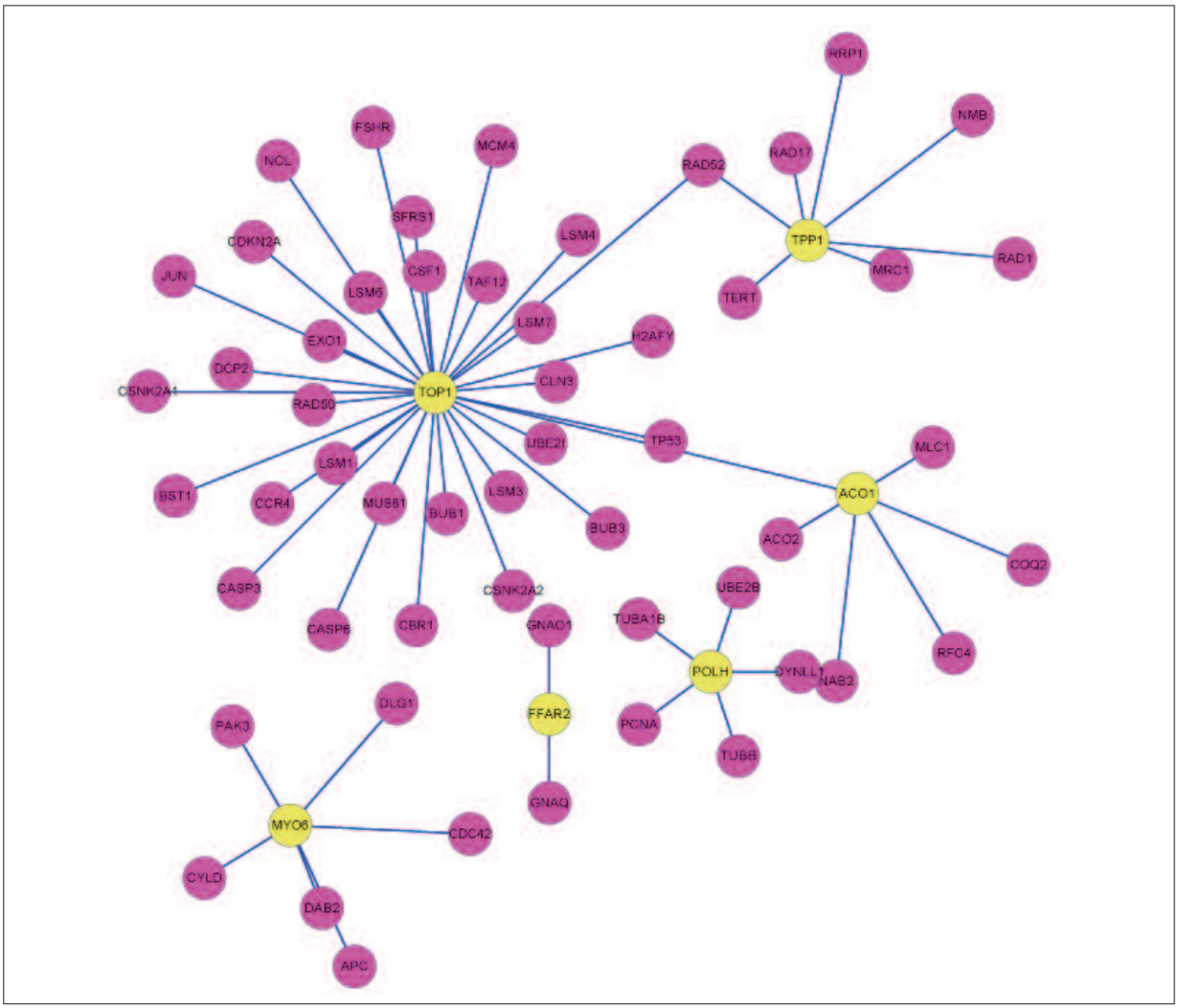

Figure 1. PPI network construction in atopic asthma. The yellow nodes stand for DEGs and the pink nodes stand for normal genes. The blue lines stand for the interaction between two proteins.

them were related to cell cycle, such as "cell cycle phase", "DNA metabolic process" "cell cycle process", "cell division" and so on. GO terms related to immunity and stress were also enriched in our network, such as "lymphocyte mediated immunity", "leukocyte mediated immunity" and "cellular response to stress" and so on.

Discussion

Asthma prevalence has increased very considerably in recent decades such that it is now one of the commonest chronic disorders in the world[2]. The prevalence of asthma poses a considerable disease burden on individuals and economic disease burden on healthcare systems and society[26]. Thus, there is an urgent need to understand the pathogenesis underlying this disease, including the protein-protein interaction networks affected during the malignant transformation process, in order to develop better detection, diagnostic and prognostic markers as well as therapeutic targets that could aid in improving clinical management and therapeutic outcome for the patients. In this present study, we identified the differentially expressed genes between healthy nonatopic samples and atopic asthmatic samples, total 12 significant changed genes were identified, including POLH, TPP1, MYO6 and ACO1 and so on.

From the result of PPI network construction in atopic asthma, we could find that many proteins have been linked by our method. The gene TOP1, TPP1, ACO1, POLH and MYO6 are hub nodes in our PPI network.

TOP1 (DNA topoisomerase 1) encodes a DNA topoisomerase, an enzyme that controls and alters the topologic states of DNA during transcription. Top1's activity is required for DNA cleavage, implying its importance in cell cycle and DNA repair. TOP1 is increasingly being recognized to be an important predictor of treatment responses for many cancers[27-29]. Leung et al[30] suggested that TOP2A may be an additional candidate gene for asthma[30]. Nonetheless, there has not been any study on the relevance of TOP1 in asthma pathogenesis. Further genetic studies with larger sample size are needed to confirm our observation.

From the result of GO enrichment analysis, we can find that most of differentially expressed genes enriched in GO terms associated with cell cycle, such as "mitotic cell cycle", "cell cycle process", and "M phase" and so on. This suggesting that cell proliferation may play an important role in the pathogenesis of atopic asthma. Our analysis is in line with previous study. Peter et al. reported that airway smooth muscle cells within the bronchial wall obtained from asthmatic patients proliferate faster in culture than those obtained from nonasthmatic patients[31]. Others have shown that bronchoalveolar lavage (BAL) from asthmatic patients contains substances that are mitogenic for airway smooth muscle and that this mitogenesis is enhanced after allergen challenge. The most widely used therapies for the control of asthma symptoms are the corticosteroids and the β_2-agonists. These anti-asthma therapies are potent inhibitors of airway smooth muscle (ASM) cell proliferation[32].

GO terms related to immunity were also enriched in our network, such as "lymphocyte mediated immunity", "leukocyte mediated immunity" and so on. Our findings are consistent with previous study that immunity plays a critical role in the pathogenesis of atopic asthma. There is considerable evidence to support a role for T cells in asthma, particularly the involvement of T_H2 cells in atopic asthma[33]. Early in 1992, Douglas et al[34] have demonstrated that atopic asthma is associated with activation in the bronchi of the interleukin-3,4 and 5 and granulocyte macrophage-colony-stimulating factor (GM-CSF) cluster, a pattern compatible with predominant activation of the T_H2-like T-cell population[34]. Several T_H2 cell-derived cytokines, IL-4, IL-5 and IL13, play a central role in asthma[35,36], particularly IL-13, which induces airway hyperresponsiveness independently of IgE and eosinophilia in animal models[33]. Antigens enter through the endobronchial tree, cross the epithelial surface, and interact with naive T_H cells and dendritic cells (DCs). As a result of signals from the surrounding microenvironment, they differentiate into T_H1 cells, which produce interferon-γ (IFN-γ), IL-2, and lymphotoxin, or T_H2 cells, which produce IL-4, IL-5, IL-9, IL-13, and IL-10. Differentiation into T_H2 cells occurs via a pathway that involves STAT-6, GATA-3, nuclear factor of activated T cells-c (NFATc), and c-maf under the influence of cluster of differentiation 8α (CD8α-) dendritic cells (DCs) and IL-4, which may come from mast cells[37].

Our PPI network is useful in investigating the complex interacting mechanisms of transcription factors and their regulated genes in atopic asthma. We also find some genes that haven't been proven to have relationship with atopic asthma; besides,

GO terms related to cell cycle and immune system have been linked by our method. However, further genetic studies with larger sample size are still needed to confirm our observation.

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