

The current genomic approaches for common respiratory diseases

Abstract

Common respiratory diseases including asthma, chronic obstructive pulmonary disease (COPD) and lung cancer are increasing worldwide. Genomic approaches for the diseases provided new insights of the mechanisms and revealed the new therapeutic targets. Genome-wide association studies (GWAS), epigenetic studies, transcriptional profiling, RNA sequencing, chromatin immune precipitation (ChIP) sequencing and microbiota investigation make personalized medicine for common respiratory diseases possible in the near future. In this review, we summarize the current progress of genomic approaches for asthma, COPD and lung cancer and also discuss the future directions of the research of the diseases.

Keywords: genomic, asthma, chronic obstructive pulmonary diseases, lung cancer

Volume 3 Issue 3 - 2016

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Received: October 26, 2016 | **Published:** November 10, 2016

Abbreviations: COPD, chronic obstructive pulmonary disease; GWAS, genome-wide association studies; ER, endoplasmic reticulum; IREs, iron-responsive elements; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; ZFN, zinc finger nuclease; RAGE, receptor for advanced glycan end products; CRISPR, clustered regularly interspaced short palindromic repeats

Introduction

The burden of chronic respiratory diseases and lung cancer is rapidly increasing worldwide. Human common respiratory diseases are complex disorders underlined by a broad range of genetic and environmental factors. Asthma and chronic obstructive pulmonary disease (COPD) are the most common respiratory diseases that are characterized by inflammation of the small airways of the lung with symptoms of wheeze and shortness of breath. The presence of inflammation can lead to airway scarring and intractable airflow limitation. Lung cancer is the leading cause of cancer death around the world. The death numbers of patients from lung cancer in America every year were greater than the death numbers of patients from prostate, breast, and colon cancer combined.¹ In recent years, with the development of genetics and genomics approaches, personalized medicine becomes more and more available for patients who have chronic respiratory diseases and lung cancers in clinic. The systemic approaches with genome-wide association studies, epigenetic studies, transcriptional profiling, RNA sequencing, ChIP sequencing and microbiota investigation brought a huge influx of data to chronic respiratory diseases and lung cancer. In this review, we briefly introduce the methodologies for genomic approaches and summarize the current progress in asthma, COPD and lung cancer, we also discuss the future research directions of the diseases.

The genomic approach methodologies

GWAS is a powerful approach for identifying the genetic causes of complex traits such as asthma and COPD. It examines the relationships

between allele frequencies and disease status with a large number of genetic markers across the genome. It is efficient to investigate the entire genome simultaneously. GWAS provides the opportunity to identify novel mechanisms of disease pathogenesis that are caused by previously unsuspected genes or regulatory regions. More than 2,000 associations for more than 300 complex diseases and traits were identified in recent years by GWASs.² The strength of GWAS is that many novel genes that were never suspected to have roles in the diseases were identified. Epigenetic effect is other possible cause of familial clustering. It regulates gene expression that is not caused by changes in the DNA sequence but by DNA methylation, histone modification and other mechanisms. The patterns of gene expression that determine cellular types and function are due to methylation of CpG sequences and gene silencing. Sex, age and other environmental factors have all been strongly associated with altered methylation in the genome. Histones are highly alkaline proteins in eukaryotic cell nuclei that package the DNA into nucleosome. Methylation, acetylation, phosphorylation, ubiquitination and sumoylation are major histone modifications. The modification can affect specific genes from activation to inactivation or vice versa. Transcriptional profiling is to quantify the transcriptional level (RNA level) of the genome in cells or tissues. There are two methods for systematic quantification of RNA in cells or tissues; one is microarray systems and the other is RNA sequencing.

The advantage for RNA sequencing is that it can possibly identify the regulation non-coding RNA in the relevant cells or tissues. Noncoding RNAs (ncRNAs) emerged as novel molecules that are important in human diseases in recent years. ncRNAs include housekeeping RNAs, long noncoding RNAs and small noncoding RNAs. Micro RNAs (miRNAs) are the most studied small ncRNAs. miRNAs are about 18-25 nucleotide long noncoding RNAs that silence target mRNAs for degradation and then inhibiting the translation. miRNAs can target 60% of mRNAs and control the signalling pathways in most cell types.³ ChIP-sequencing combines chromatin immune precipitation

with massively parallel DNA sequencing to identify the binding sites of DNA-associated proteins. Microbiota investigation is the other important field for disease research; whole microbial genome sequencing reveals the extraordinary diversity of microorganisms on Earth and their vast genetic and metabolic repertoire. The increase in accuracy, length and number of reads generated by high-throughput sequencing has coincided with a surge of interest in the human microbiota, the totality of bacteria associated with the human body, in both health and diseases.⁴

The progress of genomic approaches of asthma, COPD and lung cancer

Asthma: Asthma affects over 300 million people worldwide and is a heterogeneous disease of the airway.⁵ More than one hundred genes were found to have associations with asthma by candidate gene approaches which cannot identify novel pathways. Positional cloning is another approach that identifies disease genes by progressive dissection of linkage regions that are consistently co-inherited with the disease. *ADAM33*, *PHF11*, *DPP10*, *GPRA*, *HLA-G*, *CYFIP2*, *IRK3*, *OPN3/CHML* were discovered as asthma genes by positional cloning.⁶ Most associations identified by positional cloning studies and candidate gene studies have moderate effects in pathogenesis of asthma. The first GWAS study for asthma was carried out in the GABRIEL Consortium. The consortium identified SNPs in the chromosome 17q12-q21 region to be significantly ($P < 10^{-12}$) associated with childhood asthma and ORM1-like 3 *Saccharomyces cerevisiae* (*ORMDL3*) gene.⁷ Then a large consortium GWAS study confirmed *ORMDL3* as an important asthma suspected gene. The consortium also found *IL-18R1*, *HLA-DRB1*, *HLA-DQ*, *IL-33*, *SMAD3*, *IL-2RB*, *SLC22A5*, *IL-13* and *RORA* as asthma suspected genes.⁸ Since then, more than ten GWASs on asthma have been published. Polymorphisms of *Ch13L1*, *PDE4D*, *TLE4*, *ADRA1B*, *PRNP*, *DPP10*, *GNAI3*, *DENND1B*, *RAD50*, *IL-13*, *HLA-DR-DQ*, *LRP1B*, *SNX10*, *CA10*, *KCNJ2*, *TSLP*, *RTP2*, *PYHIN1*, *IL-12A*, *STAT4*, *IRF2*, *CDHR3*, *CTNNA3* and *SEMA3D* were found to have significant associations with asthma and asthma associated traits.^{9,10} *ORMDL3*, *GSDMB*, *TSLP*, *IL-33*, *IL-18R1*, *HLA-D*, *RAD50*, *IL-13* are the genes have been identified in multiple GWAS studies of asthma. Human airway epithelium is central to the pathogenesis of asthma. *ORMDL3*, *GSDMB*, *TSLP* and *IL-33* express in airway epithelium and they may modify the inflammatory response to epithelial damage or regulate transduction pathways that lead to asthma. *ORMDL3* influences multiple pathways in the endoplasmic reticulum (ER) that mediate inflammation during asthma. *GSDMB* may play important roles in cell proliferation. *TSLP* induces cellular senescence during airway remodelling in asthma and *IL-33* is constitutively expressed and is thought to function as an alarmin to alert the immune system after endothelial or epithelial cell damage during trauma or infection.⁶

Transcription profiling also identified interesting suspected genes for asthma. *IL-4R* was found increased in genome-wide expression profiling in allergic asthma.¹¹ *IL-5*, *IL-9* and *PRG2* expressed more in response to dust mite allergen interact with environmental in a genome wide differential gene expression study.¹² To asthma, many environmental factors such as allergens, microbes, tobacco smoke and stress influence epigenetic effects in human cells. Children growing up in a traditional farming environment had lower risk of allergic respiratory diseases. Demethylation of the *FOXP3* promoter was found in an extensive farming exposure environment.¹³ Farm childhood also had hypomethylation of *ORMDL1* and *STAT6* and

hypermethylation of *RAD* and *IL-13*.¹⁴ DNA methylation in the *CD14* promoter was also significantly less in farm mothers.¹⁵ PBMCs from obese asthmatic children had lower levels of promoter methylation of the *CCL5*, *IL-2RA* and *TBX21* and higher level promoter methylation of *TGFB1* and *FCER2*.¹⁶ Recent epigenome-wide approach identified 36 loci that had association of serum IgE level. Among them, DNA methylation events have been found in cytokine signalling genes *IL-4*, *IL-5R*, transcription factor genes *ZNF22*, *RB1*, *GATA1*, *KLF1*.¹⁷ More than 200 miRNAs have been found to associate with asthma.¹⁸ *miR-1*, *miR-21*, *miR-126*, *miR-145* and *miR-221* that have potential roles in the development of asthma through the targeting of various cytokines and transcription factors.¹⁹ For asthma, microbiota investigation found that the bronchial tree contained a characteristic flora that was disturbed by the presence of pathogens such as *Haemophilus influenzae*.²⁰

COPD: Chronic obstructive pulmonary disease (COPD) is a major cause of mortality and morbidity worldwide.²¹ Smoking is the major risk factor for COPD, but only a small portion of individuals develop COPD, indicating the genetic factors underlie the disease pathogenesis.²² The most well-known candidate genes for COPD are *CHRNA3/5*, *IREB2*, *HHIP*, *FAM13A* and *AGER*. They were replicated and confirmed in multiple populations by GWAS studies.^{23–25} None of them are targeted by treatments for COPD yet, and the mechanisms by which they alter COPD risk are still largely unknown. *CHRNA3/5* and *CHRNA4* are subunits of the nicotine cholinergic receptor. The cholinergic system is active both in cholinergic neuronal cells and in bronchial epithelial cells.²⁶ *IREB2* is a protein that binds iron-responsive elements (IREs), and is regulated in response to iron supply and oxygen. The *Ireb2* knockout mouse had abnormal iron metabolism and caused cellular dysfunction.²⁷ *HHIP* encodes a membrane glycoprotein for the Hedgehog pathway. Hedgehog signalling is important for the morphogenesis of the lung.²⁸ The RhoGAP domain of *FAM13A* may be related to COPD as Rho GTPases are key regulators of cytoskeletal dynamics, involving in the pulmonary endothelial barrier.²⁹ *AGER* is a receptor for advanced glycan end products (RAGE). The receptor belongs to immunoglobulin super family and interacts with molecules implicated in inflammation, and development.³⁰ Other candidate genes for COPD include *CYP2A6*, *RAB4B*, *MLA*, *EGLN*, *TGFB2*, *MMP12*, *RIN3*, *GSTD5*, *GSTD4*, *INTS12*, *ADAM19*, *BICD1* and *SERPINE2*.²⁶ There was little overlap in the genes that underlie asthma and the genes underlie COPD, indicating the two conditions in human airway have different disease pathogenesis.

There was evidence that cigarette smoking is associated with epigenetic in the bronchial epithelium.³¹ Epigenetic studies in lung DNA samples from COPD patients identified the COPD patients have increased *AHR* methylation, *p16* promoter methylation, *Runx3* methylation, *miR-487b* methylation, and decreased *HDAC6* methylation in lung biopsy from COPD smokers.³² In histone modification, COPD patients had increased acetylated H3 in lung that increased inflammatory gene transcription.³³ For histone modification enzymes, COPD patients had decrease *HDAC2* expression in lung tissues, alveolar macrophage and peripheral blood monocytes.^{34–36} Anti-aging and anti-inflammatory agents *SIRT1* and *SIRT6* were also found to have decreased in COPD lungs.^{37,38} Currently clinical trials on COPD epigenetic therapy focus on HDACs and the combination therapies including DNA methyltransferase inhibitors and anti-inflammatory drugs will provide a promising approach.³² A few studies identified that cigarette smoking induced a general down-regulation of miRNAs. Significantly lower expression of *miR-452* was linked to

increased expression of metalloproteinase 12 that was an important effector of smoking related diseases such as COPD,³⁹ miR-30c, miR-181d and miR-638 may also have role in regional emphysema severity and can modulate genes expression network. miR-368 may play an important roles in COPD pathology by responding to oxidative stress.⁴⁰ A recent study for miRNA screening in COPD patients found that miR-218-5p was significantly down-regulated in COPD patients, respective of smoking cessation. Pulmonary expression of miR-218-5p was mainly localized at the bronchial airway epithelium. *In vitro* and *in vivo* experiments provided functional data of miR-218-5p impacting biological pathways that were relevant to COPD pathogenesis.⁴¹ The analysis of the bacterial microbiota in COPD lung tissue confirmed that there was a significant difference in composition of the bacterial community between COPD and health lungs.^{42,43} The lungs of patients with very severe COPD contained a different community of bacteria to those of controls. These differences were largely driven by bacteria belonging to either the *Proteobacteria* or the *Firmicutes* phylum.⁴²

Lung Cancer: Lung cancer can be divided into two broad categories. Small cell lung cancer (SCLC) accounts for 15% of lung cancer cases and is a highly malignant tumour derived from cells exhibiting neuroendocrine characteristics. Non-small cell lung cancer (NSCLC) is further classified into three major pathologic subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Adenocarcinoma accounts for 38.5% of all lung cancer cases, with squamous cell carcinoma accounting for 20% and large cell carcinoma accounting for 2.9%.⁴⁴ There is a genetic component to the pathogenesis of lung cancer, whether it relates to host susceptibility to lung cancer, with or without exposure to cigarette smoke to the development of certain types of lung cancer, or to an individual's responsiveness to biologic therapies. Polymorphisms of *CYP1A1*, *GST*, *EPHX1*, *MPO*, *NQO1*, *GSTM1*, *NAT2*, *XRCC1*, *OGG1*, *ERCC1*, *XPD*, *XPA*, *XRCC3*, *p53*, *p73*, *FAS* and *FASL* were found to have association with lung cancer in many studies.⁴⁵ With the environmental stimulation, somatic mutations in genome occur in lung cells all the time. Most of the somatic mutations are harmless, but a mutation affects a gene or regulatory element and causes to a phenotypic consequence. Some of these mutations can confer a selective advantage to the cell, leading to preferential growth or survival of a clone to cancer. Such mutation can work as driver mutation for cancer.⁴⁶ High-throughput DNA sequencing has enabled the systematic sequencing of more than 2500 whole cancer genomes. This has revolutionized our understanding of the genetics of cancer, leading to the discovery of previously unrecognized cancer genes, new mutational signatures, and fresh insights into cancer evolution.⁴⁶ Lung cancer tissues sequencing revealed that mutations of *EGFR*, *KRAS*, *PIK3CA*, and *TP53* genes were important mutations for the development of lung cancer.⁴⁷ Next generation sequencing of cell-free circulating tumour DNA make it feasible to fast detection of therapeutically targetable driver and resistance mutations in lung cancer patients.⁴⁸ Acquired or epigenetic changes to DNA chromosome can also lead to increased lung cancer susceptibility.

Despite many genetic association studies, the specific genes responsible for the enhanced risk for lung cancer remain poorly understood. To understand the gene's function, particularly for the driven genes will bring new therapeutic means to treat lung cancer. Selective biological therapies have emerged to effectively treat certain types of cancers or to targetspecific determinants that are expressed by many different cancer types. Adenocarcinoma of the lung is

divided according to *EGFR* mutation, *EML4-ALK* translocation, *MET* amplification, *HER2* amplification, *FGR4* amplification and *KRAS* mutation.⁴⁴ The genotypes or expression profiling greatly influence that chemotherapy and targeting therapy of the cancers. Activating mutations in *EGFR* confer sensitivity to *EGFR* tyrosine inhibitors, but the secondary mutation T790M in exon 20 renders inhibitors ineffective.⁴⁹ The most exciting advancement in lung cancer treatment came from immunotherapy. Checkpoint blockade remove the blockade imposed by molecules such as PD1 or PDL1 on checkpoints that are required for T cell activity. The immunotherapy has proven effective around 25% of patients. Approaches to increase the response rates and identify new checkpoint molecules are on the way.⁵⁰ Clinical trials for epigenetic therapy to lung cancer are also being carried out worldwide and these investigative therapies also focusing on the epigenetic regulations on DNMTs and HDACs.³²

The future perspective of personalized medicine for respiratory diseases

The genetic and genomic approaches of asthma, COPD and lung cancer brought fruitful results for the diseases diagnosis and management in clinic. For the genes that confer the diseases, it is important to understand the functional roles in the diseases. Cellular model, mouse model and translational studies are all necessary tools for identifying the novel genes function and the pathways they regulate in human tissue and cells. Co-immuno precipitation with mass spectrum analysis is also likely to reveal the working partners with the new molecules. Proteomic approach includes liquid chromatography and tandem mass spectrometry (MS/MS) and can be used comprehensively for proteins and their biological function. The pharmacogenomics approach will apply to identify the polymorphisms of the novel genes in the search for novel drug. A knockout mouse is a genetically engineered mouse in which one or more genes have been made inoperative. A conditional knockout approach allows researchers to delete the gene of interest in a time- and space dependent manner. The technique of conditional gene expression applies site-specific DNA recombinase systems in mouse genome. The development of Zinc Finger Nuclease (ZFN) system and clustered regularly interspaced short palindromic repeats (CRISPER) system offer another effective means for genomic editing. Current epigenetic drugs for lung cancer are compounds which have off-target effects and are limiting their options for clinical trials. Single cell sequencing for lung cancer can determine the contributions of intra-tumour genetic heterogeneity in cancer development or treatment response.⁵¹ Immunotherapy for lung cancer just began. With the development of the genomic technology, it is hoped that in the near future, asthma, COPD and lung cancer will be effectively managed in the clinics with reasonably good medical care.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

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