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Genetics Association and Epigenetic Changes in COPD

Rajneesh Malhotra and Outi Vaarala

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Abstract

Genome-wide association studies (GWASs) have successfully identified susceptibility loci associated with COPD. The genes mapped on these loci, eg The FAM13A gene (family with sequence similarity 13, member A), provide a new approach to understand the COPD pathology. Furthermore, heavy smoking is reported to correlate with altered methylation and epigenetic changes of multiple genes in small airway cells. These changes have been shown to be associated with the severity of COPD. It is likely that smoking-induced changes in epigenetic control of gene expression result in genetically vulnerable individual's results in reduced tissue repair, tissue damage and persistent inflammation associated with COPD pathophysiology.

Keywords: microRNA, acetylation, GWAS, bromodomain

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by sustained inflammation of the airways, leading to destruction of lung tissue and declining pulmonary function. In COPD the repetitive challenges to the lung, due to external environmental insults, cause chronic inflammation of the bronchi and small airways leading to destruction of lung tissue (emphysema), as well as scarring (remodeling and fibrosis). The inflammatory process in COPD is characterized by predominant increase in neutrophils and macrophages in the lung and an upregulation of proinflammatory cytokines, including TNF- α , IL-1, IL-6, IL-8, IL-18, IL-17 and IL-32 [1]. Such failure to resolve inflammation and initiate proper repair causes permanent structural damage and perturbations in tissue cellular composition resulting in chronic disease [2, 3]. Smoking is the risk factor for COPD but only about 20% of smokers develop COPD [3], indicating that while smoking is an important cause or initiating factor, it is not the only driver of disease progression in COPD patients. In this chapter, we explore the

principles for the interplay between genetics and environmental factors induced epigenetic changes in the etiology and pathology of COPD pathophysiology.

2. Genetics

SERPINA1 mutation was the first fully defined genetic risk associated with chronic obstructive pulmonary diseases. SERPINA1 is the gene encoding alpha-1 antitrypsin (AAT) and systemic deficiency in AAT (AATD) due to genetic mutations can result in liver failure and chronic lung disease such as emphysema (4). AAT is the most abundant circulating serine protease inhibitor (serpin) and an acute phase reactant. The emphysema observed in patients with AATD, consisting of progressive destruction of the alveoli and small airway structure, formed the basis of the protease/anti-protease imbalance theory of chronic obstructive pulmonary disease (COPD).

Alpha-1 antitrypsin deficiency (AATD) is an inherited disorder and its role in lung emphysema was established by studying the phenotype of the patients [4]. The single candidate gene provides association for a gene in host physiology, whereas Genome-wide association studies (GWAS) identifies genome-wide set of genetic variants to a complex disease. Recently, significant progress is been made to understand the function of the genes associated with the Single nucleotide polymorphisms (SNP's) in the susceptible loci for COPD. One such COPD disease susceptibility locus is in the nicotinic acetylcholine receptor (nAChR) gene cluster CHRNA5-A3-B4 on the long arm of chromosome 15 (15q24–25.1), indicating a link to COPD is through effects on smoking behavior. nAChRs provide binding sites for nicotine and regulates nicotine mediated effects in the brain. For instance, it is been documents that smokers carrying the rs16969968 risk allele in CHRNA5 are likely to smoke more heavily than their counterparts without the risk allele. Due to increased habit of smoking these individuals run an increased risk of impaired epithelial barrier function and development of COPD due to increased smoke exposure. The CHRNA3/CHRNA5 locus has also been identified in genome-wide association studies in lung cancer and other diseases significantly influenced by cigarette smoking behavior and exposure [5, 6, 7].

Another susceptibility locus on chromosome 15 (15q25.1) is in the IREB2 gene, encoding the protein IRP2 (iron-responsive element-binding proteins), which is involved in maintaining human cellular iron metabolism. Interestingly, accumulation of iron in the lungs of healthy smokers and smokers with COPD has been reported [8], and the same has been observed in people exposed to particulate air pollutions [9]. This may be important as tight regulation of iron homeostasis is crucial not only to maintain normal cellular function, but also to prevent iron-mediated oxidative stress and damage [10]. Oxidative stress is suggested to be a key driver of pathogenesis in COPD [10], and reactive oxygen species (ROS) generated in the presence of an excess of free iron via the Fenton reaction [10] may be an important cause of cellular and tissue damage in the COPD lung.

GWAS has also identified COPD susceptibility loci on chromosome 4 in 4q22 (FAM13) and 4q31 (HHIP). FAM13A (also known as FAM13A1) is suggested to have a putative role in signal transduction in response to hypoxia. The most statistically significant SNPs are in an intronic region downstream of a Rho GTPase-activating protein (RhoGAP) domain [11], COPD risk alleles at

the FAM13A locus is reported to be associated with increased expression of FAM13A in human lungs. In a recent publication it was reported that in murine and human lungs, FAM13A is expressed in airway and alveolar type II epithelial cells and macrophages [12]. Furthermore, the authors reported that Fam13a null mice (Fam13a2/2) were resistant to chronic cigarette smoke-induced emphysema compared with Fam13a expressing mice. It was shown that FAM13A modulates catenin pathway by interacting with protein phosphatase 2A and recruits protein phosphatase 2A to regulate glycogen synthase kinase β activity resulting in β -catenin degradation. These results indicate that FAM13A could increase the susceptibility of mice to emphysema development by inhibiting β -catenin signaling. β -catenin signaling activates the expression of Wnt target genes that promote cell proliferation and limit cell differentiation [11]. In the absence of FAM13A, β -catenin pathway is restored leading to a more effective lung repair program in lung alveolar epithelial cells after CS exposure. This hypothesis was further supported by expression data, indicating increased FAM13A protein levels and decreased β -catenin protein levels in human COPD lung tissues compared with ex-smokers without COPD [11, 12].

These observations provide evidence for potential role for these pathways in COPD patients with emphysema and potential for utilizing SNP's associated with FAM13A for patient stratification for drugs modulating catenin pathway. Increased levels of FAM13A blocks the pathways associated with lung regeneration, therefore inhibition or deletion of FAM13A in COPD patients with risk allele at the FAM13A locus could be a potential way to induce epithelium regeneration in emphysematic patients.

In two independent genome-wide association studies, Hedgehog interacting protein (HHIP) was identified as a COPD susceptibility gene in subjects from the Framingham Heart Study and a homogenous case control cohort from Norway, respectively [13, 14]. The SNPs map to an intergenic region upstream of the HHIP gene and regulates HHIP gene expression in carriers. HHIP is expressed in epithelial cells and considered to modulate epithelial barrier structure as reported by Zhou et al. [15]. Zhou and workers [15] reported that modulation of HHIP expression results in differential expression of 296 epithelial genes, most of which associated with ECM matrix interactions, junctional complexes or cell growth. Analysis of epithelial cells derived from human lung tissue showed significantly decreased expression of these genes in cells from COPD patients as compared to cells from healthy controls. These observations indicate loss of HHIP function in human bronchial epithelial cells may contribute to susceptibility to smoke-induced COPD by regulating genes important for epithelial barrier integrity and function [15].

In a recent meta-analysis study, Busch and coworkers [16] genotyped 3346 single nucleotide polymorphisms (SNP) in 2588 cases (1803 severe COPD) and 1782 controls from four cohorts. They analyzed association testing with COPD, combining their results with existing genotyping data from 6633 cases (3497 severe COPD) and 5704 controls. In this analysis, authors confirmed significance of previously documented SNPs in the Transforming growth factor beta 2 (TGFB2), FAM13A, HHIP, Matrix metalloproteinases 3 (MMP3)/Matrix metalloproteinases 12 (MMP12), and CHRNA3/CHRNA5/IREB2 regions with COPD pathophysiology. Authors identified two SNPs at loci not previously identified. These two loci showed significant associations between SNPs near PPIC and PPP4R4/SERPINA1 and severe COPD.

The GWAS analysis has indicated genes associated in COPD pathophysiology. In the future further investigation of specific pathophysiology regulated by genes associated

with SNP's may allow patient stratification based on genetic linkage and development of drugs to modulate disease phenotype.

3. Epigenetics of COPD

Epigenetics is defined as study of heritable changes in gene expression resulting in change in cellular phenotype caused by mechanisms that do not alter the Deoxyribonucleic acid (DNA) sequence. Epigenetic modifications control gene expression by changes to the structure and function of chromatin. Environmental challenges such as cigarette smoke, can lead to permanent changes in epigenetic patterns (epimutations) resulting in development of chronic disease. Examples of such epigenetic mechanisms include DNA methylation, histone modification and RNA interference [17].

DNA methylation is a process resulting in the covalent addition of a methyl group to cytosine residues part of cytosine-guanine (CpG) dinucleotides, and when a gene is methylated on CpG islands in promoter areas, transcriptional repression is generally the result, representing an important mechanism for gene silencing. In patients with COPD some loci are identified as hypermethylated and other hypomethylation [18–20]. The loci identified to be hypermethylated are reported to be Genes components of the PI3K/Akt and anti-oxidant NFE2-related factor-2 (Nrf2) pathways [20]. The CpG hypermethylation observed in the PTEN and NFE2L2 (Nrf2) genes correlates with reduced expression of their respective gene products. The Nrf2 pathway is anti-inflammatory and protects against oxidative stress, whereas PTEN is a negative regulator of PI3K/AKT signaling, contributes to inflammation, remodeling, and proteolysis in COPD. Thus, CpG hypermethylation appears to antagonize protective pathways regulating pathophysiology of COPD. On the other hand, hypomethylation of the HDAC6 promoter has been linked to elevated expression of HDAC6 in COPD [21]. HDAC6 is thought to contribute to cigarette smoke-mediated epithelial dysfunction in COPD by promoting autophagy [21].

Posttranscriptional modifications to histone ends in the histone H3 and H4 residues define the accessibility of the chromatin to the different coactivators or corepressors. Histone acetylation is regulated by the levels and activities of histone acetyl transferases (HAT) and histone deacetylases (HDAC). Increase in HAT activity results in increased acetylation of lysine residues in histones and gene transcription, whereas as increase in HDAC activity negatively regulates gene transcription. Simplistically, chromatin is transcriptionally active when lysines on histones H3 and H4 are acetylated.

It has been proposed that the inhibition of HDAC2 activity also contributes to the glucocorticoid resistance seen in COPD inflammation [22–24]. In lung biopsies obtained from COPD patients reduced HDAC2 activity and increased acetylation of histones have been reported [25]. The reduced HDAC2 expression and activity is shown to be associated with increased acetylation of histones H2A, H2B, H3, and H4 in the lungs and alveolar macrophages of COPD patients [26]. Yang et al. [27] reported that chronic cigarette smoke (CS) results epigenetic modifications of histone causing abnormal and sustained lung inflammatory response that occurs in smokers and in patients with COPD. They reported increased levels of KC, MCP-1, IL-6, and GM-CSF in mouse lung homogenate at both 3 days and 8 weeks of CS

exposure in mice. Furthermore, they demonstrated using Chromatin immunoprecipitation (ChIP)-sequencing in CS exposed mouse lung that pro-inflammatory gene expression was due to the increased phosphorylation/acetylation of specific histone H3 (lys9/ser10) and histone H4 (lys12) in the promoter region of pro-inflammatory gene. Chen et al. [28] reported that cigarette smoke decreased the levels of HDAC1 expression and increased H3K9 acetylation. These modifications were associated with altered expression of pro-inflammatory mediators in CS-induced rat lungs and in macrophages.

These reported altered activity of HDAC's with increased histone acetylation in preclinical models and alveolar macrophages from COPD patients points to a potential role for epigenetic pathways in chronic lung inflammation in COPD patients.

Taken together these studies indicate that an imbalance between histone acetylases and deacetylases contributes to disease specific alterations in gene expression.

Sirtuin-1 (SIRT1) expression and deacetylase activity are reduced in peripheral lung tissues from patients with COPD [29, 30]. Reduced SIRT1 activity correlated with increased expression of MMP9 and H4 pan-acetylation [29]. Recently, Baker et al. [24] reported that oxidative stress (hydrogen peroxide) induces reduction of SIRT1/–6 in bronchial epithelial cells (BEAS2B). SIRT1 is reported to inhibit autophagy, cellular senescence, fibrosis, and inflammation by deacetylation of target proteins using NAD⁺ as co-substrate. Similarly SIRT6 have also been shown to be associated with reduction–oxidation reaction (redox) state and inhibits cellular senescence and fibrosis [31]. Therefore, pathways associated with activation of SIRT1 and SIRT6 are an attractive approach for novel therapeutic targets for COPD. In the preclinical models efficacy of non-selective activators of SIRT1 using the pharmacological activator SRT1720 has been demonstrated. Activation of SIRT1 via SRT1720 inhibits pulmonary neutrophil accumulation, and completely restored exercise tolerance and the fall in oxygen saturation and protects against cigarette smoke (CS) and elastase-induced emphysema in mice [32]. Furthermore, resveratrol, a substance shown to activate SIRT1 attenuates cigarette smoke extract (CSE)-mediated glutathione depletion through reversing CSE-mediated NRF2 carbonylation in lung epithelium cell line, A549 cells [31, 32]. Due to a recent study in human airway smooth muscle cells, resveratrol is suggested as an anti-inflammatory therapy alternative to corticosteroids in COPD, particularly in COPD exacerbations [33].

The family of acetylation binding module termed as bromodomain recognizes acetylation sites on chromatin and was identified in the early 1990s in the brahma gene from *Drosophila melanogaster* [34]. The human proteome encodes 61 bromodomains, which are present in 46 diverse nuclear and cytoplasmic proteins. The BET (bromodomain and extra-terminal) proteins (BRD2, BRD3, BRDT and BRD4) belong to this family of bromodomain containing proteins. BET proteins bind to acetylated lysines in the histones of nucleosomal chromatin and function either as co-activators or co-repressor of gene expression [34]. Due to the hyperacetylation patterns observed in COPD the modulation of BET (bromodomain and extra-terminal) proteins are new targets in modulating pathophysiology in patients. Nicodeme et al. [35] reported that a synthetic compound I-BET, a potent inhibitor of bromodomain-containing BET proteins to acetylated histones, dissociates the formation of the chromatin complexes essential for the LPS-induced expression of inflammatory cytokines in a temporal manner (early middle and late response). Recently, we proposed that inhibition of BET protein interactions with

hyperacetylated sites in the chromatin will regulate excessive activation of pro-inflammatory genes and survival of inflammatory cells associated with inflammation in COPD. We demonstrated for the first time a known BET inhibitor, JQ1, showed a difference potency for inhibitions of IL6 in human peripheral blood mononuclear cells (PBMC) from normal or COPD in comparison to alveolar macrophages stimulated with LPS [31]. Furthermore, BET inhibitor JQ1 attenuated multiple genes, including pro-inflammatory cytokines and genes regulating innate and adaptive immune cells. We have used the set of genes modulated by JQ1 to generate a gene signature defining BET regulated genes [34]. Analysis of the expression of these gene signature genes, 10 genes - CMPK2, EPSTI1, IFI44, IFI44L, IFIT1, IFIT3, MX1, OAS2, RSAD2 and XAF1, in the COPD samples compared to samples from normal controls, a subset of COPD patients with increased expression of these signature genes were identified. These results indicate that epigenetic modification associated gene expression could be utilized to do patient stratification and identify patients likely to show maximum response to given epigenetic modulator.

Nimmo and coworkers [36] have reported the co-localization of differentially methylated CpG regions and predisposing SNPs identified by GWAS in Crohn's disease. They observed methylations within 50 kilobases from several GWAS susceptibility loci, including IL-27, IL-19, TNF, MST1, and NOD2. A similar analysis in COPD is yet to be conducted. An analysis of epigenetic modification of genes identified by GWAS in COPD, including low probability associations, could highlight key pathways and points of regulation involved in the disease pathology, enabling target identification, patient stratification and prediction of treatment response.

4. MicroRNA-silencing RNA

A microRNA (miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, that regulates the expression of complementary RNA thus functions in RNA silencing and post-transcriptional regulation of gene expression [37]. The expression of miRNAs are generally downregulated in smokers compared to non-smokers in airway epithelium [38], alveolar macrophages [39] and in lung tissue [40]. Dicer is an enzyme that cleaves pre-microRNA (pre-miRNA) into microRNA [41]. It is proposed that smoking downmodulated Dicer activity following sumoylation of Dicer resulting in downmodulation of miRNA in smokers [41]. Microarray-based studies have identified a large number of differentially expressed miRNAs in primary fibroblasts and lung tissue from patients with COPD [40–42]. Many of the miRNAs implicated in COPD have also been associated with various cancers, whereas miRNA that discriminated COPD from lung cancer have also been identified, including miRNAs 26a, 641, 383, 940, 662, 92a, 369-5p, and 636 [43] and miRNAs 20a, 24, 25, 152, 145, 199a [43]. Reduced expression of miR-199a-5p was reported in regulatory T cells from patients with COPD. miR-199a-5p targets genes includes members of the TGF β superfamily [44] therefore plays a role in T-regulatory cells (Treg) cell function. Reduced miR-199a-5p expression in regulatory T cells in COPD may enhance regulatory T cell function resulting in abnormal bias towards Th1 immune responses [44]. Therefore, dysregulated miRNAs are potential treatment targets in COPD although additional studies are warranted to help determine whether observed differences in miRNAs are a cause or consequence of COPD.

5. Conclusion

The chronic exposure to cigarette smoke or other air pollutants possibly induce epigenetic modifications, changes in lung cellular microenvironment and epithelial dysfunction. This may pathogens and to drive structural damage and changes in tissue cellular architecture typical of chronic inflammatory disease. Further research is required to map the specific epigenetic changes in lung tissue obtained from COPD patients, and combine this data with a detailed analysis of the disease pathophysiology to identify specific targets and patient stratification biomarker for modulating symptoms in COPD.

Author details

Rajneesh Malhotra* and Outi Vaarala

*Address all correspondence to: rajneesh.malhotra@astrazeneca.com

Immunity Department, RIA IMED Biotech Unit, AstraZeneca Gothenburg, Mölndal, Sweden

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