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Epigenetic biomarkers for lung cancer and copd

Sarika Pandey[1]*, Rajiv Garg[2], Surya Kant[3], Priyanka Gaur[4], Mohammad Waseem[5]

[1]PhD, Department of Respiratory Medicine, King George's Medical University, Lucknow- 226010, Uttar Pradesh, India

[2]Professor, Department of Respiratory Medicine, King George's Medical University, Lucknow- 226010, Uttar Pradesh, India

[3]Professor&Head, Department of Respiratory Medicine, King George's Medical University, Lucknow- 226010, Uttar Pradesh, India

[4]PhD, Department of Physiology, King George's Medical University, Lucknow- 226010, Uttar Pradesh, India

[5]PhD, Department of Biochemistry, King George's Medical University, Lucknow- 226010, Uttar Pradesh, India

Correspondence Author: Sarika pandey, Department of Respiratory Medicine, King George's Medical University, Lucknow- 226010, Uttar Pradesh, India

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Abstract

Lung cancer and Chronic Obstructive Pulmonary Disease (COPD) are the leading causes of morbidity and mortality worldwide. Development of lung cancer and COPD involves both genetic and environment factors. Due to lack of an effective screening method for early detection of lung cancer, patients are diagnosed at a very advanced stage. Analysis of DNA methylation biomarkers is an emerging field that provides promising potential for improving the clinical process of lung cancer diagnosis as well as COPD. DNA methylation biomarkers provide a range of opportunities for early detection, diagnosis, prognosis, therapeutic stratification and post-therapeutic monitoring.

Keywords: Lung Cancer, COPD, DNA methylation, Epigenetics

Introduction

Lung cancer and Chronic Obstructive Pulmonary Disease (COPD) are the leading causes of morbidity and mortality worldwide. Although the cause of most lung cancer is well known, the disease has proven difficult to diagnose early and treat successfully, reflecting limited advances in our understanding of the molecular mechanisms underlying lung carcinogenesis and individual susceptibility to lung cancer. Imaging and cytology-based screening strategies have been employed for early detection, and while some are sensitive, none have been demonstrated to reduce lung cancer mortality. [1] Development of lung cancer and COPD involves both genetic and environment factors. Cigarette smoking is the major environmental risk factor for lung function decline and COPD. In addition to genetic alterations, epigenetic mechanism is closely involved in pathogenesis of lung cancer. Characterized by an abnormal persistent inflammatory response to noxious environmental stimulation, COPD has shown to increase the susceptibility for lung tumorigenesis in previous research. Current research on epigenetics of lung cancer and COPD has focused on aberrant DNA methylation, histone acetylation and non-coding RNAs

regulation. Apart from mutational changes, epigenetic changes also have an important role in cancer development. Due to lack of an effective screening method for early detection of lung cancer, patients are diagnosed at a very advanced stage. Studies on molecular biomarkers occurring at early stages of disease is immense need for proper treatment and management of disease as early detection offers the opportunity for therapeutic intervention..

Biomarkers from various sources such as genetics, proteomics, and epigenetic approaches are in use for clinical research purposes and have a great potential for improving the management of lung cancer & COPD in clinical routine[2-4].The analysis of DNA methylation biomarkers provides promising potential for improving the clinical process for diagnosis of lung cancer as well as COPD. Epigenetic alterations are innovative biomarkers for cancer due to their stability and non invasive accessibility in bodily fluids. As these epigenetic modifications are also reversible they can be potentially useful as therapeutic targets [5].

Recently DNA methylation has emerged as a prime source of potential cancer-specific biomarkers. Typically this occurs in CpG rich regions called CpG islands at/near gene promoters. These islands are ~200-1,000 bp in length. In cancer, many genes become hypermethylated. In addition to general hypomethylation of the genome, hypermethylation of CpG islands in gene promoter regions occurs in cancer cells [6]. Methylation often results in the silencing of tumor suppressor or growth regulatory genes [7]. Methylation plays an important role in normal cells as well as in tumor development. In normal cells it contributes to chromatin organization, silencing of transposable elements, X chromosome inactivation, tissue-specific expression and genetic imprinting [8, 9]. There is a key role of DNA methylation and mutation events in airway epithelial cells in the early development of COPD. ssome molecular alterations in DNA are produced by reactive oxidant species which are found in tobacco smoke [10, 11]. The tobacco specific nitrosamine 4 NNK which is a precursor of the alkylating agents leads to the methylation of guanosine residues in DNA [12]. The inflammation in the lung airway due to these molecular alterations may contribute to COPD pathogenesis and can predispose individuals towards the development of LungCancer [13, 14]. Most of the patients with lung cancer suffer from non-small cell lung cancer (NSCLC) [15].

Aberrant DNA methylation is a common phenomenon in human cancer. DNA methylation biomarkers can be helpful in early detection, diagnosis, prognosis, therapeutic stratification and post-therapeutic monitoring. Study by Zhang Y et al[16] showed that, nine genes (APC, CDH13, KLK10, DLEC1, RASSF1A, EFEMP1, SFRP1, RAR β and p16(INK4A)) demonstrated a significantly higher frequency of methylation in NSCLC compared with the normal tissues ($P \leq 0.001$), the aberrant promoter methylation of the tumor suppressor genes , p16,[17] H-cadherin, [18] death-associated protein (DAP) kinase 1 (DAPK1) [19] RASSF1A[16], tissue inhibitor of metalloproteinase 3 (TIMP3), O6-methylguanine-DNA-methyltransferase (MGMT), E cadherin (ECAD), p14ARF and glutathione S-transferase P1 (GSTP1) in primary non-small cell lung cancers[20] are few most studied genes in lung cancer. As per Mikesha T et al [21] currently the most promising methylation biomarkers are CDKN2A and SHOX2 in lung cancer. Previous work in Chilean subjects by methylation specific PCR (MSP), demonstrated a high prevalence of CDKN2A promoter methylation in squamous cell lung carcinomas (SQCs) and adenocarcinomas (AdCs)[22,23]Methylation of the CDKN2A promoter CpG Island has been observed in the sputum of subjects at risk for lung cancer 3 years prior to diagnosis[24]. Hypermethylation of the CDKN2A exon 2

CpG island observed in colorectal and bladder cancers [25, 26]. The detection of CDKN2A methylation in a high fraction of plasma samples of lung cancer patient is a non-invasive detection [27]. Previous study show association of DNA methylation of CDKN2A with poor survival in adenocarcinoma and NSCLC patients The promoter region of the CDKN2A gene was found to be hypermethylated in 61.1% of tumor samples in colorectal cancer. CDKN2A is prone to hypermethylation during lung cancer development. In another previous study alteration of CDKN2A in NSCLC adenocarcinoma tissue samples was noted as 38% (17/45) and included 10 homozygous deletions, 4 methylations and 3 mutations [30]

The strongest evidence supporting early methylation of CDKN2A is the observation that methylation of this gene can precede clinical diagnosis of lung cancer. Two studies independently report the detection of CDKN2A in sputum of individuals with no detectable cancer [31] CDKN2A methylation was evident in two sputum samples which had been collected from subjects almost three years prior to diagnosis. [32]. The CDKN2A a tumour suppressor gene involved in susceptibility to malignant melanoma [33] familial pancreatic cancer [34] and in breast cancers [35]. Somatic mutations of CDKN2A are present in tumors of various sites including head and neck tumors [36] squamous cell carcinoma of the larynx [37],colon cancer[38],clear cell sarcoma [38],and respiratory tract tumors [39]. Methylation of SHOX2 has also emerged as a biomarker for diagnosis of lung cancer .Previous study by Schmidt B et al[40] found SHOX2 methylation in 62% of bronchial aspirates which were found negative by cytological analysis and in Another study by Kneip C et al[41] on SHOX2 methylation in plasma samples, found a sensitivity of 60% and a specificity of 90% for detecting lung cancer .

Genetic and epigenetic aberrations of CDKN2A can lead to enhanced tumorigenesis and metastasis and thereby recurrence of cancer. As per study by Zhao et al [42] in these cases, the restoration of genetic and epigenetic reactivation of CDKN2A will be the practical approach for the prevention and therapy of cancer. Brock MV et al [43] studied (CDKN2A, CDH13, RASSF1A and APC) 4 genes associated with early recurrence in stage I non-small-cell lung carcinoma. Other gene that is frequently silenced by promoter methylation is the DNA repair gene O6-methylguanine- DNA-methyltransferase (MGMT) [44]. It is a DNA repair enzyme that protects cells from the carcinogenic effects of alkylating agents by removing the adducts from the O6 position of guanine. Thus, the p16 and MGMT genes are strong candidate biomarkers for early detection. Using a highly sensitive PCR approach to detect methylated DNA sequences, re Palmisano et al. [45] reported that methylation of p16 and/or MGMT could be detected in DNA from sputum in 100% of patients with squamous cell lung carcinomas up to 3 years before clinical diagnosis. Decrease in MGMT expression has been seen in some tumor tissues, and lack of activity in some cell lines. Loss of expression is rarely due to deletion, mutation, or rearrangement of the MGMT gene, but methylation of discrete regions of the CpG islands of MGMT [46, 47] has been associated with the silencing of the gene in cell lines [48]. MGMT plays a crucial role in the defence against alkylating agents that generate 06-alkylguanine in DNA, a major trigger of genotoxicity and apoptosis. Screening MGMT expression levels in tumors and normal tissue of the individuals will be helpful in predicting efficacy of methylation based cancer therapies [49-51]. Among more than 500 primary tumors studied, MGMT hypermethylation was reported in a subset of specific type of cancer [52]. Aberrant methylation has been seen in nearly 40% of the tumors in gliomas and colorectal carcinomas, [53], whereas in non-small cell

lung carcinomas, lymphomas, and head and neck carcinomas, this alteration has been found in 25% of the tumors [54]. Liu Yet al [55] in their study showed a higher frequency of promoter methylation for the p16 and MGMT genes in lung tumors from smokers compared with never-smokers, indicating an association between tobacco use and the increased incidence of promoter methylation of these genes in lung cancer.

Huang t et al [56] identified significant associations between seven genes (CDKN2A, RASSF1, MGMT, RARB, DAPK, WIF1 and FHIT) and smoking behaviour in non-small cell lung cancer patients. CDKN2A hypermethylation was a common risk factor of smoking behaviour in NSCLC patients, however, RARB hypermethylation was only found as a risk factor of smoking in Chinese but not in other populations. p16, DAPK, PAX5b, and GATA5 has been considered as potential biomarkers for NSCLC in sputum and serum samples [57]. Integrated analysis of (miR-31 and miR-210 and DNA methylation biomarkers RASSF1A and 3OST2 in sputum has a synergistic effect for lung cancer early detection[58] . CDH1 is also frequently methylated in lung carcinomas [**59]** .The cadherins are a family of cell-surface glycoproteins responsible for homophilic cell recognition and adhesion [60]. Several family members, including CDH1 (E-cadherin) and CDH13 (H-cadherin), are located on the long arm of chromosome 16, a region of frequent allelic loss in lung cancers.

COPD is an independent risk factor for lung carcinoma, particularly for squamous cell carcinoma [61] and the high prevalence of lung cancer in COPD suggests that there may be common mechanisms, such as premature aging in the lungs, genetic predispositions to either disease or common pathogenic factors, such as growth factors, activation of intracellular pathways or epigenetics [62]. Cigarette smoking has been considered as a most important risk factor for COPD and lung cancer and there are increasing evidences that links these two diseases beyond a common etiology. Study by Sundar et al [63] suggests that DNA methylation in suggestive genes, such as NOS1AP, BID, and GABRB1 may be used as epigenetic signatures in smokers and patients with COPD if the same is validated in a larger cohort. Future studies are required to correlate DNA methylation status with transcriptomics of selective genes identified in this study and elucidate their role and involvement in the progression of COPD and its exacerbations. 349 CpG sites has been significantly associated with the presence and severity of COPD [64] . Gene ontology analysis based on these 349 CpGs (330 genes) suggested the involvement of a number of genes responsible for immune and inflammatory system pathways, responses to stress and external stimuli, as well as wound healing and coagulation cascades. They concluded in their study that genetic and epigenetic pathways may both contribute to COPD. In another study by Tessema M et al, [65] the reduced expression of CCDC37 and MAP1B associated with COPD likely predisposes these genes to methylation that in turn, may contribute to lung cancer..

Most of the preclinical and clinical experience in lung cancer with epigenetic therapy has been focused on NSCLC.[66. The combination of DNMT inhibition with HDAC inhibition was found to have a greater proapoptotic effect than monotherapy [67-68].Epigenetic modifications are also reversible and potentially useful as therapeutic targets. 5-azacytidine and 5-aza-2´-deoxycytidine are demethylating drugs that have been approved by the US FDA for treatment either as single agents or in combination with other drugs, for the treatment of other blood cancers and solid tumors [69]**.** Assessing the effect of therapies with demethylating drugs will be helpful in near future for management of these diseases**.**

Conclusion

Epigenetic studies might shed new insights into the pathogenesis of COPD susceptibility and severity. Identification of epigenetic biomarkers using the safest, least invasive and affordable method may be of use in future for early detection, primary prevention and treatments of lung cancer and COPD patients. As epigenetic modifications are also reversible they are potentially useful as therapeutic targets. research for accurate epigenetic biomarker is needed to minimize the treatment burden which many cancer patients face with untargeted therapies and its related serious side effects .The study on epigenetic biomarkers will be helpful in early diagnosis of COPD and lung cancer patients and focus on demethylation therapy will be helpful in future treatment of these patients and improving their quality of life.

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