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Expression of GULP1 in bronchial epithelium is associated with the progression of emphysema in chronic obstructive pulmonary disease

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ABSTRACT

Background: GULP1 functions as a cytoplasmic adaptor protein involved in the engulfment of apoptotic cells. The aim of this study was to investigate the expression and/or promoter methylation of GULP1 in the bronchial tissue and the lung parenchyma of chronic obstructive pulmonary disease (COPD) patients and control subjects without COPD (non-smokers and smokers).

Methods: Using a case–control design, we selected a group of 15 subjects with non-small cell lung carcinoma (NSCLC), 15 subjects with COPD, 9 subjects of without COPD (4 non-smokers and 5 smokers) as controls. We studied the expression of GULP1 in the bronchial tissue and the lung parenchyma by means of immunohistochemistry (IHC). To understand the mechanistic aspect of GULP1 expression in COPD and NSCLC, we performed quantitative methylation specific PCR (QMSP) in cases and controls of COPD and NSCLC.

Results: Our IHC analysis revealed that GULP1 was not expressed in pneumocytes or alveolar macrophages of COPD patients, however, GULP1 expression was detected at different levels in bronchial epithelium. GULP1 expression statistically correlated with severity of COPD-emphysema ($p = 0.001$, χ^2 test). GULP1 promoter methylation was not observed by QMSP assay in any of the samples thereby excluding the role of promoter methylation in differential expression of GULP1 in COPD and NSCLC.

Conclusions: This study provides preliminary observations on the differences in GULP1 expression in different cellular components of lung tissues from COPD and control subjects. Our findings suggest a potential role for GULP1 in the pathogenesis and progression of COPD-emphysema that warrants further investigation.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of deaths in the USA and projected to be the third biggest killer in the world by 2020 [1]. COPD is characterized by irreversible airflow limitation, which is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. The typical symptoms of COPD are dyspnea, chronic cough and/or sputum production and diagnosis should be confirmed by spirometry in patients who have

Abbreviations: AM, alveolar macrophage; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FFPE, formalin-fixed paraffin-embedded; FVC, forced vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; H & E, hematoxylin & eosin; IHC, Immunohistochemistry; NSCLC, Non-small cell lung cancer; QMSP, quantitative methylation specific PCR.

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symptoms. The most commonly encountered risk factors for COPD are cigarette smoking and inhalation of polluted air resulting from burning of wood and other biomass fuels [2].

DNA methylation, which is one of the main epigenetic events, is considered to have a 'causal' role in the molecular pathophysiology of smoking-related lung diseases [3–5]. Recent reports suggested that promoter methylation of several genes, including CDKN2, MGMT, FUT7, and SERPINA1 are involved in the development of COPD. Moreover, promoter methylation of some of these genes plays a key role in both the presence and severity of COPD [3,5–8]. Smoking can lead to epigenetic alteration of numerous genes. As COPD is mainly a smoking related disease, it is expected that several genes and pathways will be altered in COPD by changes in expression and promoter methylation.

A key event in the phagocytosis process is 'eat-me' signals. 'Eat-me' signals are specifically displayed on the surface of apoptotic cells, but not on healthy cells, for selective clearance of dying cells by phagocytosis [9]. The apoptotic cell engulfment is characterized by complex steps involving several genes such as GULP1, ELMO, Rac, and DOCK. Defects in apoptotic cell clearance play a role in a wide variety of human pathologies including COPD [9–12]. Alveolar macrophages (AMs) remove potentially pathogenic microorganisms via phagocytosis and are essential for maintenance of the normally sterile environment within the lung. Chronic airway inflammation and continuous tissue repair environment lead to the development of an altered AM population [13,14]. A study utilizing AMs from COPD patients showed reduced phagocytosis of common airway pathogens [14] increasing susceptibility to infection and repeated pulmonary exacerbations in COPD subjects.

GULP1 has been recently identified as a key player in phagocytosis of apoptotic cells [15,16] and impaired functioning of GULP1 is reported in several diseases (e.g., arthritis, schizophrenia) [17,18] including cancer [19–21]. GULP1 is reported to be ubiquitously expressed in professional phagocytes (i.e., macrophages) as well as in a wide variety of human tissues including lungs [15]. An epigenetic unmasking study from our lab showed significant methylation in the GULP1 promoter of several primary tumors and cell lines [20,21].

COPD and non-small cell lung carcinoma (NSCLC) are two of the most common pulmonary diseases caused by cellular damage in response to smoke exposure [22,23]. Analysis of GULP1 promoter methylation in these two diseases will add important mechanistic insight into our understanding of these diseases. To this end, the first objective of this study was to compare GULP1 promoter methylation in lung tissues from COPD patients, control group without COPD (non-smokers and smokers), and NSCLC patients. Secondly, we sought to look at GULP1 protein expression in various cell types in the lung tissue. To our knowledge this is the first study to investigate the epigenetics and expression of GULP1 in lung tissues from COPD-emphysema, control group, and NSCLC patients.

2. Material and method

2.1. Patients and tissue specimens

We obtained lung tissue specimens from 15 COPD patients, 9 control groups without COPD (4 non-smoker and 5 smoker) and 15 NSCLC patients. All patients had adequate formalin-fixed, paraffin-embedded (FFPE) tissue blocks. NSCLC samples were obtained from patients who underwent surgical resection at The Johns Hopkins University School of Medicine, and COPD patients and control samples were obtained from Medical School, University of Crete, Greece. COPD severity was defined in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification based on post-bronchodilator forced expiratory volume in 1 s (FEV_1) in patients with FEV_1/forced vital capacity (FVC) < 0.7 of preoperative spirometry [2]. Our study included 5 GOLD I (mild COPD-emphysema), 5 GOLD II (moderate COPD-emphysema), 4 GOLD III (severe COPD-emphysema) and 1 GOLD IV (very severe COPD-emphysema) patient samples.

Approval for research on human subjects was obtained from The Johns Hopkins University institutional review board. This study qualified for exemption under the U.S. Department of Health and Human Services policy for protection of human subjects [45 CFR 46.101(b)].

2.2. Cell lines

All of the cell lines used in this study were cultured accordingly to the recommendations of the American Type Culture Collection (Manassas, VA, USA), from where they were purchased. The details of cell lines are shown in Supplementary Table 1.

2.3. Gene selection for promoter methylation

COPD is characterized by chronic inflammation of the airway epithelium producing debris in the microenvironment of this pathological condition. If these debris can be cleared in time, progression of COPD could be delayed. As noted above, we recently identified an engulfment gene (GULP1), whose silencing leads to progression of cancer. Based on our hypothesis that silencing of engulfment pathway genes may lead to impaired clearance of debris and progression of COPD, we selected 8 genes (GULP1, ELMO1, ELMO2, ELMO3, BAI-1, BAI2, BAI3 and MUC5B) for sequencing based promoter methylation analysis in a small set of COPD tissue samples. Based on our initial findings and availability of GULP1 primers and probes from our cancer studies, we analyzed extended numbers of COPD and NSCLC samples by quantitative methylation specific PCR (QMSP).

2.4. DNA extraction

Histological slides were obtained from FFPE tissues. All original histologic slides were reviewed to reconfirm the diagnosis by a senior pulmonary pathologist. A representative FFPE block that contained sufficient amount of tissue was retrieved for DNA extraction and several 10 μm slides were obtained from each block. The presence of tumor cells was confirmed by staining the first and last slides of the representative block with hematoxylin & eosin (H&E). The tumor samples were micro-dissected to obtain >70% of neoplastic cells. DNA was extracted from cell lines and slides prepared from FFPE blocks using the phenol-chloroform extraction protocol followed by ethanol precipitation as described previously [24].

2.5. Bisulfite treatment

2ug of DNA extracted from primary tumors and cell lines was subjected to bisulfite treatment, which converts unmethylated cytosine residues to uracil residues, as described previously [25]. EpiTect Bisulfite kit (Cat No. 59104, from QIAGEN Inc. Valencia, CA – 91355) was used for this conversion, following the manufacturer's instructions.

2.6. Quantitative fluorogenic methylation specific PCR (QMSP)

DNA obtained from primary human lung tissue specimens (15 COPD patients, 9 control groups without COPD and 15 NSCLC patients) and 15 lung cell lines were used for QMSP analysis of GULP1. The summary of the samples and outlines of methods used in this study is summarized in Fig. 1.