行政院國家科學委員會專題研究計畫成果報告

急性呼吸窘迫症候群 BAL 及肺組織之黏液醣蛋白及其基因之表現(I)

Mucin and mucin gene expression in BAL and lung tissue of patients with ARDS

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一、中文摘要

呼吸道之黏液醣蛋白是分子量相當大（>200 KD**）的醣化蛋白，覆蓋於呼吸道上皮細胞之表面，有保護和潤滑的作用。而急性呼吸窘迫症候群(**ARDS**)是肺部相當嚴重且廣泛的傷害，由於各種發炎細胞及介質的作用，造成肺泡及肺泡微血管破壞。病人呈現呼吸衰竭，且有極高的死亡率。但一直沒有好的生物標記來預測急性呼吸窘迫症候群的發生及預後。我們先前的研究發現，急性呼吸窘迫症候群病人血清中，含有比正常人（吸菸、非吸菸群）、慢性支氣管炎患者、心因性肺水腫病人較高的黏液醣蛋白相關抗原，且血清黏液醣蛋白值與** ARDS **病人之肺傷害指標有相當好之相關性，而** ARDS **病人血清中大分子之黏液醣蛋白的比例減少，被分解之小分子黏液醣蛋白增加。所以我們設計這個計畫，利用特異單株抗體免疫化學染色評估肺組織中黏液醣蛋白的含量及分佈。想進一步證實急性呼吸窘迫症候群之肺組織中黏液醣蛋白特異基因的表現型態，以了解在發炎反應對黏液醣蛋白基因表現的影響。**

關鍵詞：黏液醣蛋白、急性呼吸窘迫症候群

Abstract

Mucins are high molecular weight glycoproteins (MW>200kD) present in the human airway with the function of protection and lubrication. Acute respiratory distress syndrome (ARDS) represents a severe, diffuse lung injury caused by a wide variety of insults. The overwhelming inflammatory cells and mediators result in disruption of the alveolocapillary membrane, and leakage of a protein-rich fluid into the alveolar airspaces. Our previous study showed serum levels of mucin-associated antigen elevated in patients with ARDS. Serial measurements of serum mucin levels were inversely correlated with static respiratory system compliance and log (PaO_2/FiO_2), and positively correlated with lung injury score. The serum mucins of patients with ARDS were polydispersed and smaller than the antigens in normal sera. We hypothesize that elevated serum mucin is due to increased production, increased permeability or increased degradation to smaller molecules before or after they gain access into blood stream. In this project, we used immunohistochemical stains to evaluate mucin expression in lung tissue of patients with ARDS in comparison with the values of control groups. We want to compare the pattern of mucin genes expression in ARDS and normal lung tissue; as a basis to study the mucin genes expression in inflammation.

Keywords: Mucins, Acute Respiratory Distress Syndrome

二、背景與目的

The acute respiratory distress syndrome (ARDS) represents a severe, diffuse lung injury caused by a wide variety of insults, such as sepsis, aspiration, pneumonia, multiple trauma, multiple transfusion, pancreatitis and others. Studies estimated the incidence of ARDS to be 5.2 to 7.1 cases per 100,000 per year. Although the sequence of events leading to ARDS is complex, a final common pathway involving a variety of inflammatory cells and mediators ultimately results in disruption of the alveolocapillary membrane. The permeability of pulmonary endothelium and epithelium increases with loss of macromolecular barrier. The pathophysiology of ARDS is still under vigorous studies. Most of the previous studies of markers for ARDS addressed on markers of acute inflammation in BALF or plasma. However, a complex interplay between many pro-inflammatory and anti-inflammatory mediators obscures these studies. A marker for measurement of integrated acute inflammation and cell injury may be helpful.

Mucins are major components of airway mucus secreted by bronchial epithelial mucous cells (goblet cells and submucosal glands). Mucins are complex glycoproteins with high molecular weight (MW > 200 kD) and heavy glycosylation (> 70% carbohydrate by weight). The glycosylated side chains are mainly α-1,3-O-glycosylated (>80%), which distinguishes them from those of other common glycoprotein. The human respiratory mucins belong to a broad family of different mucin peptides. So far, several distinct gene loci of mucins have been identified in human. Some well-known genes have been named MUC1, -2, -3, -4, -5B, -5AC, -6, -7 and -8. MUC1, -2, -4, -5B, and -5AC are reported to be the major mucin genes expressing in the lung and airway. MUC3, -6, -7 are not expressed in the lung.

We used an ELISA method with a human airway mucin specific monoclonal antibody (17Q2) to measure serum mucin levels in normal subjects, chronic smokers, patients with chronic bronchitis and other pulmonary diseases, patients with acute cardiogenic lung edema, and those with ARDS. These results suggest that serum mucin antigen from acute lung injury may either result from increase in production or protease cleavage before or after they gain access into blood stream. It is anticipated that airway mucin production is increased in patients with ARDS, however, no direct proof has been known.

To define the hypersecretion of mucin in patients with ARDS, we measured the mucin expression in lung tissue of ARDS patients by immunohistochemistrical studies in comparison with those of control groups. We used 17Q2 monoclonal antibody to detect the carbohydrates of secretory airway mucin expression, HMFG2 (a monoclonal antibodies against MUC1 core proteins) to detect MUC1 , and 45M1 (an antibody

against MUC5AC core proteins) to detect MUC5AC .

Specific Aim

1. To determine whether mucin expression increased in lung tissue of patients of ARDS.
2. To determine which subtype of airway mucin production is increased in patients of ARDS.

三、方法與結果

I. Lung tissue collection:

Twenty consecutive patients with ARDS received lung biopsy and twenty patients of lung cancer received surgical resection were enrolled. Immediately after operation, lung tissue from patients of ARDS or uninvolved part of lung cancer specimen were collected. A piece of lung tissue will be fixed in formalin and paraffin embedded. The non-tumor parts of surgical specimens of lung cancer from 20 nonsmokers were used as the control group.

II. Tissue Immunohistochemistry

Immunohistochemical analysis is performed on lung tissue derived from open lung biopsy specimens obtained from ARDS patients and non-tumor part of tissue from patients with lung cancer. The paraffin blocks were sectioned at a thickness f 4 to 6 μm. Sections were dewaxed and rehydrated, treated for 10 min with 3% (vol/vol) H_2O_2 in 18%(vol/vol) methanol in 0.1 mol/L phosphate buffer containing 0.9% (wt/vol) NaCl, pH 7.4 to block endogenous peroxidase activity, washed three times in PBS, and treated with 1% normal goat serum for 30 min at room temperature. After a brief washing, sections were placed flat in a humidified box and incubated with monoclonal antibody for 30 to 45 in at room temperature. The sections were washed thoroughly in PBS after each primary antibody incubation. Sections were subsequently incubated wtih1:150 biotinylated goat antimouse immunoglobulin for 45 min, and then with 1:150 streptavidin-horseradish peroxidase for 30 min. Peroxidase activity is detected with 3,3'-diaminobenzidine as a chromogen, with H_2O_2 as a substrate. Sections are counterstained with hematoxylin. Positive control specimens are from lung adenocarcinoma known to express a high level of mucoprotein. The stains are examined by two investigators and classified into 4 grades (0 to 3) according to the proportion of positivity (Grade 0 = no positive epithelial cells; Grade 1 = less than 10% positive epithelial cells; Grade 2 = 10 to 49% positive epithelial cells; and Grade 3 =50 to 100% positive epithelial cells). Any block given a different score by the examiners was reviewed with a video monitor for final grading by both reviewers.

III. Mucin immunohistochemical staining with 17Q2

Expression of mucin detected by 17Q2 was increased in alveolar lining epithelia (most are degenerative type II pneumocytes) and bronchiolar epithelia. There were cytoplasmic and membranous staining.

In patients with diffuse fibrosis, the lining epithelia of lung cysts did not express 17Q2-detected mucin. In control normal lung tissue, the alveolar epithelium did not express 17Q2-detected mucin. It implied mucin expression increased in alveolar epithelia in lung tissue of patients with ARDS.

IV. Mucin immunohistochemical staining with HMFG2 and 45M1

Expression of mucin detected by HMFG2 (MUC1) was increased in alveolar lining epithelia (most are degenerative type II pneumocytes) and bronchiolar epithelia. There were cytoplasmic and membranous staining.

In patients with diffuse fibrosis, the lining epithelia of lung cysts did not express HMFG2-detected mucin. The staining pattern of 17Q2 and HMGF2 were similar. In control normal lung tissue, the alveolar epithelium did not express HMFG2-detected mucin.

In control normal lung tissue and lung tissue of patients with ARDS, the alveolar epithelia did not express 45M1-detected mucin.

These implies that the increased mucin expression in alveolar epithelia of patients with ARDS is caused by increased MUC1 but not MUC5AC.

四、參考文獻

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