Evidence of Two Ciliated Epithelial Cell Subsets in Mouse Airways

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Abstract

Patients with asthma-associated airway epithelial damage exhale increased levels of nitric oxide (NO). However, the distribution of endothelial NO synthase (eNOS) in mouse airways remains to be controversial. In the present study, we present preliminary immunofluorescent staining data help clarify the contribution of ciliated cells to epithelial maintenance in the airways.

Keywords:
Mouse airway; Ciliated cells; eNOS; Scgb1a1; Naphthalene

Introduction

Nitric oxide (NO), an important messenger molecule in cells, is formed endogenously in the airways of the lung. NO generated by various isoforms of NO synthase (NOS) plays crucial and diverse physiological roles and has been implicated in several diseases including asthma and chronic obstructive pulmonary disease [1]. Endothelial NOS (eNOS), originally identified in the endothelium of vascular tissue, is responsible for the majority of the NO produced in this tissue [2]. NO that is produced by eNOS acts as a bronchial vasodilator [3]. In addition to its expression in vasculature tissues, eNOS has been found in the epithelia of airways and is important for their functions. For example, eNOS localizes to the basal membrane of ciliary microtubules in rat lungs and stimulates ciliary beat frequency, which determines the functions of the ciliated cells [4,5]. However, it remains controversial whether ciliated cells contribute to epithelial regeneration in the airways.

Ciliated cells are a terminally differentiated population that is replenished by club cells and/or stem cells residing in the airway epithelia under steady state or during lung injuries [6,7]. Several studies have shown that ciliated cells are capable of giving rise to goblet cells under certain conditions. For example, the epidermal growth factor receptor is activated in ciliated cells during Sendai virus infection, leading to the expression of goblet cell markers in these cells [8]. Turner and colleagues have provided evidence that human ciliated cells convert to goblet cells in culture [9]. In these studies, ciliated cells are characterized by only a few markers including acetylated tubulin (ACT) and Forkhead box protein J1 (FoxJ1). Thus, two or more functionally distinct ciliated cells possibly exist in the airway epithelia, and they cannot be distinguished using the ACT or FoxJ1 markers. In this study, we present preliminary immunofluorescent staining evidence showing that the ciliated cells of the mouse airway include two subsets that are distinguished by their expression of eNOS. These data help clarify the contribution of ciliated cells to epithelial maintenance in airways.

Materials and Methods

Animals

C57BL/6 mice were maintained in pathogen-free conditions in the Tianjin animal facility. Mice were exposed to a 12-h light/dark cycle and had free access to food and water. Adult mice between the ages of 2 and 4 months were sacrificed for experiments according to a protocol approved by the Nankai University Animal Care and Use Committee.

Reagents

Naphthalene, mouse IgG2b anti-ACT (1:8,000) antibody, and goat polyclonal anti-Scgb1a1 antibody (1:50) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mouse monoclonal IgG1 anti-eNOS (1:500) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

Naphthalene Administration

Naphthalene was dissolved in Mazola corn oil and injected intraperitoneally at a dose of 275 mg/kg. All the injections were performed between 8:00 and 10:00 a.m. to normalize injury responses.
Untreated animals were used as controls. At the day post-naphthalene injection indicated, the mice were sacrificed for analysis.

Immunofluorescence Staining

Lung lobes were processed for histological analysis by using standard methods described previously [10]. Briefly, lung tissues were fixed by instillation of 10% neutral buffered formalin, followed by immersion in 10% neutral buffered formalin for a total of 2 h at 4°C. Five-micrometer sections were collected from the lung tissues. Sections were incubated with primary antibodies at 4°C overnight, washed with phosphate-buffered saline, and then incubated with the appropriate fluorochrome-conjugated secondary antibody for 2 h at room temperature. Slides were mounted in Fluoromount G containing 4',6-diamidino-2-phenylindole (DAPI). Staining was visualized using a Zeiss Axiovert40 inverted fluorescent microscope.

Results

Expression of eNOS in mouse airway epithelia

To assay eNOS expression in mouse airways, immunofluorescence staining was performed on lung tissue sections. Immunoreactivities of Scgb1a1 and eNOS indicated that they colocalized in the proximal airway, suggesting that eNOS is expressed in mouse club cells (Figure 1 A–C). Club cells negative for eNOS were not observed in the present study. Consistent with the previous reports [4,11], the immunoreactivities of eNOS and the ciliated cell marker ACT revealed that ciliated cells in the proximal airway expressed eNOS (Figure 1 D–F). We also observed for the first time that the proximal airway epithelium in the mouse contained a subset of ciliated cells that did not express eNOS (Figure 1 D–F). These eNOS-negative ciliated cells were also observed in the distal airway epithelium (Figure 1 G–J). Analysis of 3–5 individual lung sections indicated that eNOS-positive ciliated cells were more abundant than eNOS-negative ciliated cells in both the proximal and the distal airways of mouse (Figure 1K).

eNOS-negative ciliated cells resist naphthalene-induced lung injury

Ciliated cells that express eNOS have been shown to be resistant to naphthalene-induced lung injury [12]. Therefore, we examined whether eNOS-negative ciliated cells had a similar capacity. To address this, naphthalene was injected intraperitoneally into mice. As reported previously [13], most Scgb1a1-expressing club cells were ablated 1 day post-naphthalene (Figure 2A), except for a few Scgb1a1-expressing cells residing in the distal airway that survived the naphthalene-induced injury. These surviving Scgb1a1-expressing cells were immunoreactive for eNOS (Figure 2B). Moreover, we observed that both eNOS-positive (Figure 2C–D) and eNOS-negative ciliated cells (Figure 2C–D) survived naphthalene injury. By day 10, during the post-injection repair process, a significant number of Scgb1a1-expressing cells were regenerated in the airway epithelium (Figure 2F–I). During the injury process (day 1 post-naphthalene), greater than 70% of the surviving ciliated cells in both the proximal and distal airways were negative for eNOS (Figure 2J). However, during the repair process (day 10 post-naphthalene), the relative abundance of eNOS-positive ciliated cells in the total ciliated cell population returned back to normal levels in both the proximal and the distal airways (Figure 2K).

Further, more alveolar cells exhibited immunoreactivity for eNOS in the naphthalene-injected group compared to the controls, suggesting that the expression of eNOS can be induced by naphthalene injury.
by a naphthalene-resistant epithelial stem cell population that has been altered and unable to repair itself. NO is an important messenger in this process, the airway epithelium of asthmatic patients appears to be impaired [19]; however, Konig and colleagues found that club cells are not progenitors [15]. During the airway reparative process, club cells are characterized recently [18]. Club cells are known to express eNOS [16,17]. Hierarchically, club cells are replenished in the distal airway. Reciprocal tissue repair and potential to become ciliated and mucous cells have also been proposed to serve as progenitors of goblet cells. In vitro culture of human ciliated cells that express enhanced green fluorescent protein (eGFP) in the presence of the asthmatic cytokine interleukin [13] leads to the eGFP labeling of goblet cells, suggesting that ciliated cells have the potential to give rise to goblet cells [9]. However, recently reported data indicated that this is not the case in mouse airways. Genetic labeling of ciliated cells with enhanced yellow fluorescent protein indicated that ciliated cells proliferate but do not give rise to goblet cells in the ovalbumin-induced murine model of allergic lung disease [27]. In the current study, we observed for the first time that the two subsets of ciliated cells exist in mouse airways that show differential expression of eNOS. One subset expresses eNOS, but the other one does not. Both subtypes survived naphthalene-induced lung injury. We were unable to distinguish their functions in the current study, but our data raise a number of interesting questions regarding the potential roles of the ciliated cells in airway maintenance and repair. For example, it is not clear which one subtype of ciliated cells give rise goblet cells under asthmatic conditions. Therefore, future efforts to isolate these two cell subtypes and to identify their functions in steady state and reparative processes after lung injuries are crucial to address these questions.

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References


Discussion

The airway epithelium plays critical roles as a protective physical and functional barrier between the external environment and underlying tissues, and as a central element in the initiation and regulation of immune responses in the lung [14]. Epithelial repair is initiated quickly after injury by club cells, which act as endogenous progenitors [15]. During the airway reparative process, club cells exhibit differential potential to become ciliated and mucous cells [16,17]. Hierarchically, club cells are replenished in the distal airway by a naphthalene-resistant epithelial stem cell population that has been characterized recently [18]. Club cells are known to express eNOS [19]; however, Konig and colleagues found that club cells are not immunoreactive for eNOS in rat lungs [20]. This discrepancy might be attributed to the source of the eNOS antibodies used in the different studies. Our data clarify this issue by showing that eNOS clearly colocalized with Sgb1a1, a marker of epithelial club cells.

A large body of evidence indicates that structural and functional epithelial alterations play critical roles in both the development and persistence of asthmatic inflammation [21]. During this pathological process, the airway epithelium of asthmatic patients appears to be altered and unable to repair itself. NO is an important messenger in cells and increased levels of exhaled NO are detected in patients with bronchial asthma [22,23]. Incomplete repair of the airway epithelium in asthmatic patients suggests epithelial regeneration may be negatively regulated by NO. Studies performed using an ovalbumin-induced murine asthma model showed that a 75% decrease in club cells and a 25% decrease in ciliated cells are accompanied by a marked increase in mucin-producing goblet cells [24].

Cilia are an important instrument of mucociliary clearance [25]. NO stimulates ciliary beat frequency and, therefore, is beneficial to airway mucociliary clearance [26]. Loss of ciliated cells contributes to the reduced ability of mucociliary clearance in asthma. Ciliated cells have also been proposed to serve as progenitors of goblet cells. In vitro culture of human ciliated cells that express enhanced green fluorescent protein (eGFP) in the presence of the asthmatic cytokine interleukin [13] leads to the eGFP labeling of goblet cells, suggesting that ciliated cells have the potential to give rise to goblet cells [9]. However, recently reported data indicated that this is not the case in mouse airways. Genetic labeling of ciliated cells with enhanced yellow fluorescent protein indicated that ciliated cells proliferate but do not give rise to goblet cells in the ovalbumin-induced murine model of allergic lung disease [27]. In the current study, we observed for the first time that the two subsets of ciliated cells exist in mouse airways that show differential expression of eNOS. One subset expresses eNOS, but the other one does not. Both subtypes survived naphthalene-induced lung injury. We were unable to distinguish their functions in the current study, but our data raise a number of interesting questions regarding the potential roles of the ciliated cells in airway maintenance and repair. For example, it is not clear which one subtype of ciliated cells give rise goblet cells under asthmatic conditions. Therefore, future efforts to isolate these two cell subtypes and to identify their functions in steady state and reparative processes after lung injuries are crucial to address these questions.

References


