

# การศึกษาลักษณะรูปร่างสามมิติของเซลล์บุผิวในหลอดลมของ สัตว์เลี้ยงลูกด้วยนม ด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด

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## บทคัดย่อ

วัตถุประสงค์ในงานวิจัยนี้เพื่อศึกษาลักษณะของเซลล์บุผิวในหลอดลมซึ่งมีโครงสร้างที่สอดคล้องกับหน้าที่ พบว่าลักษณะของเซลล์บุผิวประกอบด้วยเซลล์หลัก 3 ชนิดคือ ciliated cell, non-ciliated cell และ goblet cell โดยที่ ciliated cell เป็นลักษณะของ cell ที่มี cilia ยาวปกคลุมเป็นจำนวนมาก เพื่อต่อการทำหน้าที่ในการพัดโบกสิ่งแปลกปลอมที่เข้ามาในทางเดินหายใจ และพบว่ามี goblet cell จำนวนมากที่ช่วยในการหลั่งสารเมือก ช่วยให้ secretion จับตัวกันเป็นก้อนและอาศัยการพัดโบกของ cilia ขับ secretion เหล่านี้ออกมา เพื่อไม่ให้ลงไปหลอดลมส่วนที่อยู่ลึกลงไป ส่วน non-ciliated cell ถูกปกคลุมด้วย microvilli ซึ่งเชื่อว่ามีบทบาทเกี่ยวข้องกับการควบคุมปริมาณของ secretion จากการศึกษาครั้งนี้เป็นการศึกษาถึงเซลล์บุผิวที่เป็นเซลล์หลักทั้งสามชนิด แสดงความสอดคล้องกันในลักษณะรูปร่างของเซลล์บุผิวแต่ละชนิดที่สัมพันธ์กับหน้าที่ในระบบทางเดินหายใจ และแสดงถึงภาพโครงสร้างของเซลล์บุผิวในเชิงสามมิติได้ชัดเจน

คำสำคัญ: หลอดลม เซลล์ที่มีซีเลีย เซลล์ที่ไม่มีซีเลีย เซลล์หลั่งสารเมือก จุลทรรศน์อิเล็กตรอนชนิดส่องกราด

## **Surface topography of tracheal cells of common tree shrew (*Tupaia glis*)**

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### **Abstract**

The surface morphology of the luminal surface of tracheal epithelial cell was demonstrated by mean of scanning electron microscopy. The trachea was investigated that the ciliated cells, non-ciliated cells, and goblet cells (mucous cells) were clearly observed in three dimensions. The ciliated cell provided long cilia to protect lung from foreign body. The goblet cells which lined the mucous membrane were also over the cilia and entrapped foreign body in the inspired air. This mucus substance was fused the particles and was propelled by cilia outward. It led to protect lung from invasion. The non-ciliated cells or microvillus cells were covered with short microvilli. They were believed that the microvilli surface were important in regulate the volume of the tracheobronchial secretion. Our results confirm that the morphology of each cell was related to the function for conducting airway.

Key words: trachea, ciliated cell, non-ciliated cell, goblet cell, scanning electron microscopy

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## Introduction

The lung was the major organ of gas exchange. As medical interest in the effect of air pollution increase, however, the study of basic structure and function of respiratory epithelium of lung was remained to elucidate. The tracheobronchial pathway was exposed to pollutants or infectious agents derived from the external or internal environment.

In the structure of lung, the conducting respiratory airway was composed of trachea, primary bronchi, extrapulmonary tract, secondary bronchi, bronchioles and terminal bronchioles. The structure of bronchi was similar to that of the trachea. When they entered the lungs (intrapulmonary tract), the cartilage ring were replaced by irregularly shaped cartilage plates, which completely surround the bronchi.

In the tracheobronchial lining, it was a tall-columnar, pseudostratified ciliated columnar epithelium in which at least 13 cell types; 11 epithelial and 2 mesenchymal cells have been identified by transmission electron microscopy<sup>1</sup>. Not all of these cells were found in every species, and most of the epithelium consisted of ciliated and non-ciliated cells, those which elaborated the surface mucous layer. It has been reported that intraepithelial corpuscles (chemoreceptor<sup>2</sup>) were found along the length

of the conducting airways.

As an important function, the membrane lining larger airways was the production of secretory which covers the luminal surface. Non-ciliated epithelial cells in mammalian conducting respiratory airways were divided into 3 types: mucous (goblet) cells, serous epithelial cells, and Clara cells. Mucous and serous were considered to be the secretory cells of the most distal conducting airways or bronchioles. In 1983<sup>3</sup>, Charles G. reported that mucous cells were observed in tracheal epithelium of many species such as hamster, rat, rabbit, cat, Bonnet monkey and sheep, while serous cells were only presented in rat.

It is known that Common tree shrew (*Tupaia glis*) is one of animal model for mammal in various fields such as comparative anatomy, phylogeny and evolution. This species is different from rodent but it is primitive primate<sup>4</sup>.

In this study, we have investigated the surface characteristics of tracheal epithelial of Common tree shrew (*Tupaia glis*) using scanning electron microscopy (SEM). Furthermore, the present study was important, add to the existing knowledge of the respiratory epithelium, particular of the main epithelial cells in mammalian trachea.

## Materials and methods

Five adult male Common tree shrew (*Tupaia glis*) injected intraperitoneally with 50 mg/kg of pentobarbital sodium. Average of weight was  $280 \pm 25$  gm. The animals were incised by midline thoracotomy. The respiratory tract were removed and fixed by airway infusion of 2.5% glutaraldehyde fixatives with 0.1 sodium cacodylate, pH=7.4. The trachea and primary bronchus were collected stored in the same fixative until processing. The specimens were cut into pieces perpendicular to the long axis. The pieces were washed in 0.2 M cacodylate buffer, and post-fixed in 1% osmium tetroxide. Therefore, they were dehydrated in ethanol and dried with critical point drying techniques. These specimens were coated with gold in an ion-sputtering apparatus. The epithelial structures were observed in a Jeol, JSM 5400 operating at 15 KV.

## Results

Scanning electron micrographs revealed the epithelial cells lining the luminal surface of the trachea and primary bronchus (Fig. 1). The dominant epithelial cells composed of ciliated cells and non-ciliated cells were found in the luminal surface of these airways (Fig. 2). Some non-ciliated cells or microvillus cells contain

mucous granules as designated goblet cells. These cells were covered with short microvilli which processes a prominent filamentous coat. Mucous secretion was extruded from mucous cell or goblet cell. The boundary of non-ciliated cells was look like polyhedral shape (Fig. 2). The non-ciliated cells were covered with short microvilli that exposed the apical surface. Sometime these cells were designated as microvillus cells. The lateral surface of ciliated and non-ciliated cells was revealed obviously by scanning electron microscopy. The mucous granules were protruded on the apical surface as shown in the fig. 3, 4, and 5. The lateral sides of goblet cells were shown. The fig. 6 and 7 was shown clearly the depth of the lateral side of the cells.

The numerous ciliated cells were densely covered with tall cilia approximately  $5 \mu\text{m}$  in length, and  $0.3 \mu\text{m}$  in diameter. The shaft was bathed in secretion, while the tips of cilia penetrate the overlying mucus layer (Fig. 8, 9, 10).

The young or immature cilia were beginning to emerge. It was processing of cilliogenesis as shown as in fig. 11.

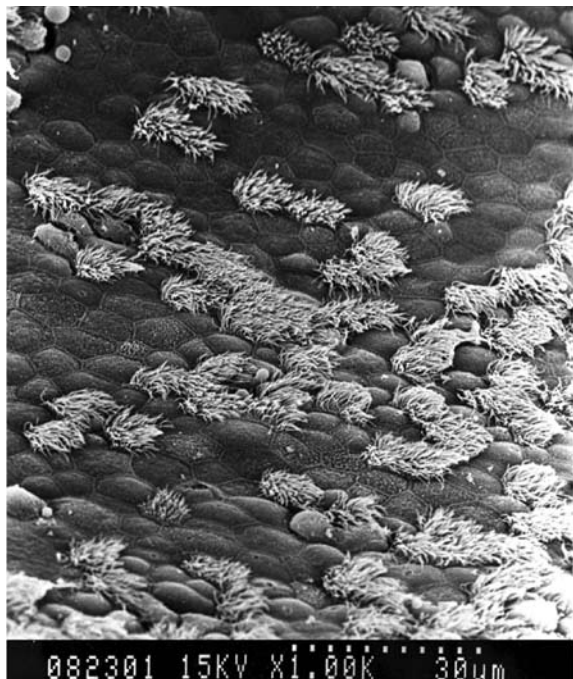


Figure 1 Low magnification of scanning electron micrograph showed tracheal cells. They found in the luminal surface.

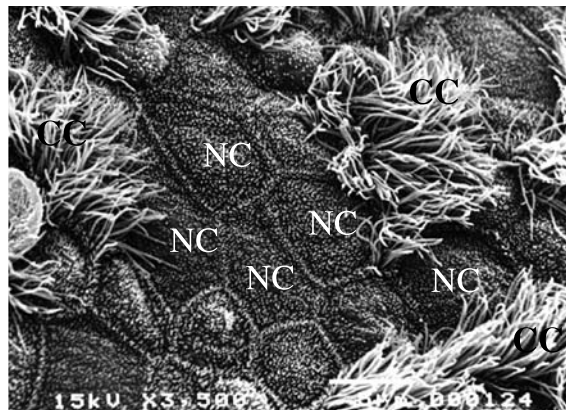


Figure 2 Higher magnification of scanning electron micrograph; two dominant cells: ciliated cell (CG) and non ciliated cell (NC). The ciliated cells (CC) were densely covered with cilia. The non-ciliated cell (NC) was covered with microvilli. The boundary of each non-ciliated cell looked to be polyhedral shape.



Figure 3 The ciliated cell (CC), non-ciliated cell (NC) and goblet cell (GC) were observed. The mucus secretion (\*) was extruded from goblet cell (GC) or mucous cell.

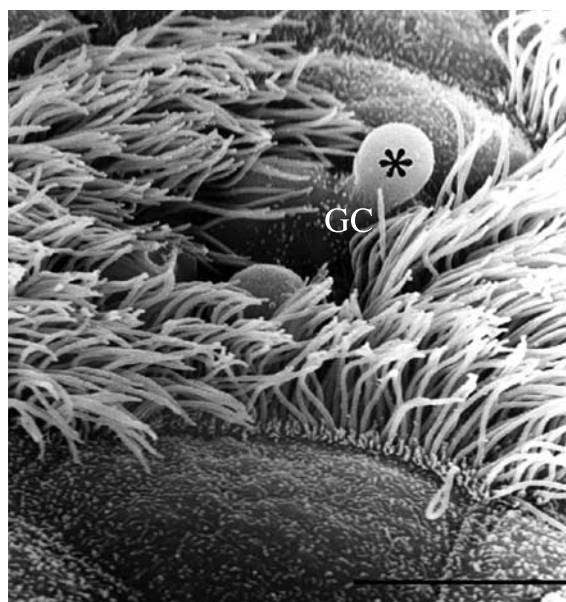


Figure 4 Higher magnification from figure 3. Some stages in the releasing of mucin (\*) from the goblet cells was clearly. X 8,000, bar = 5  $\mu$ m.

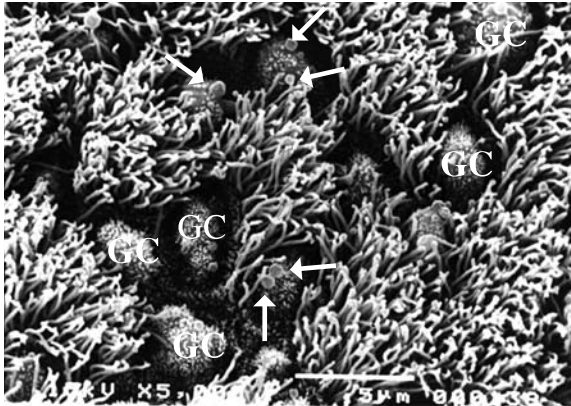


Figure 5 Goblet cells (GC) were dispersed between ciliated cells. Some stages in the releasing of mucin (arrow head) from goblet cells.

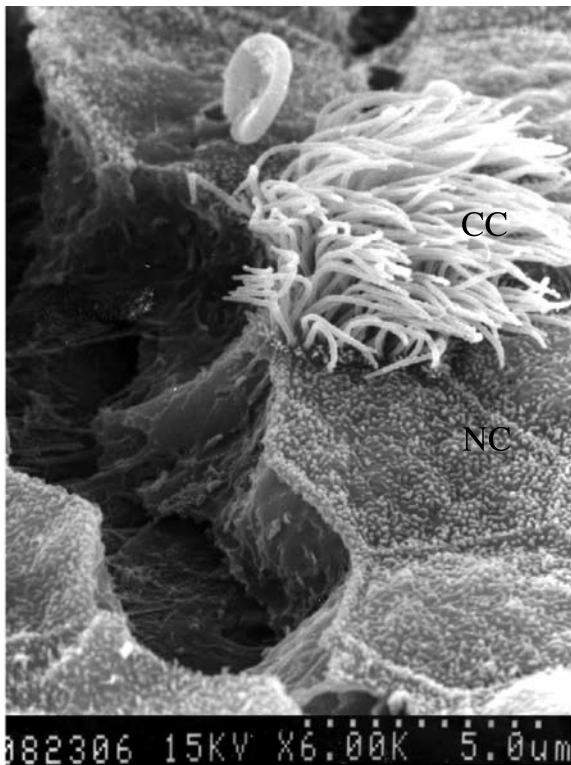


Figure 7 Higher magnification of the lateral side of ciliated (CC) and non-ciliated cell (NC). The boundary of the cells was clearly observed.

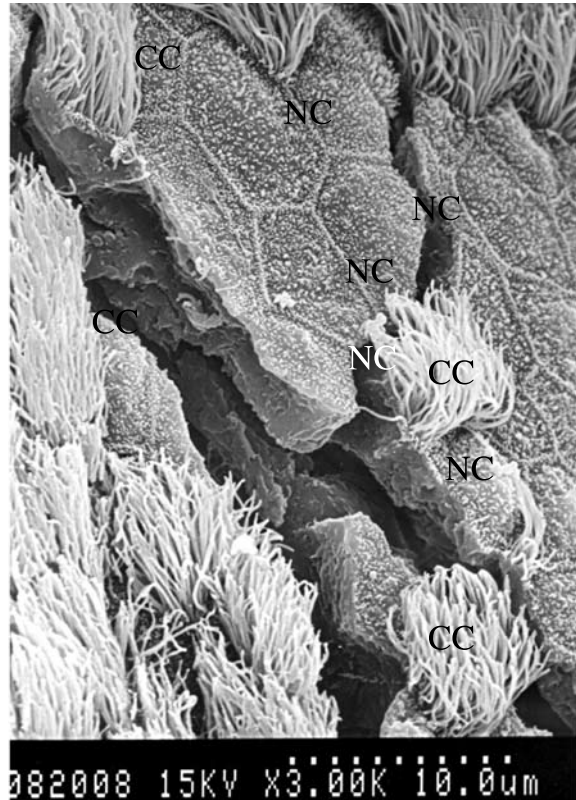


Figure 6 The lateral surface of ciliated (CC) and non-ciliated cells were obviously in three dimensions.

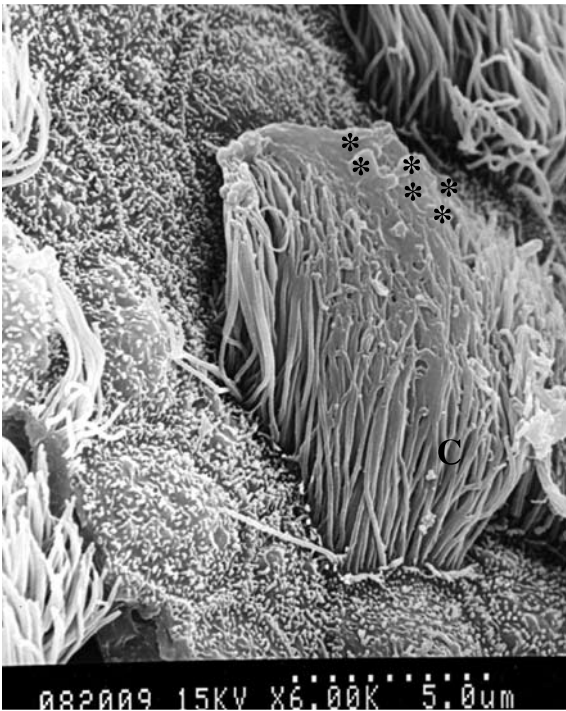


Figure 8 The mucous substance lines over the cilia (C) because of its sticky nature, entrap that foreign body in the inspired air. The mucin (\*) contain foreign body that was propelled by cilia outward, thereby preventing the entrance of foreign body lungs interior. Thus, cilia protect lung from invasion.

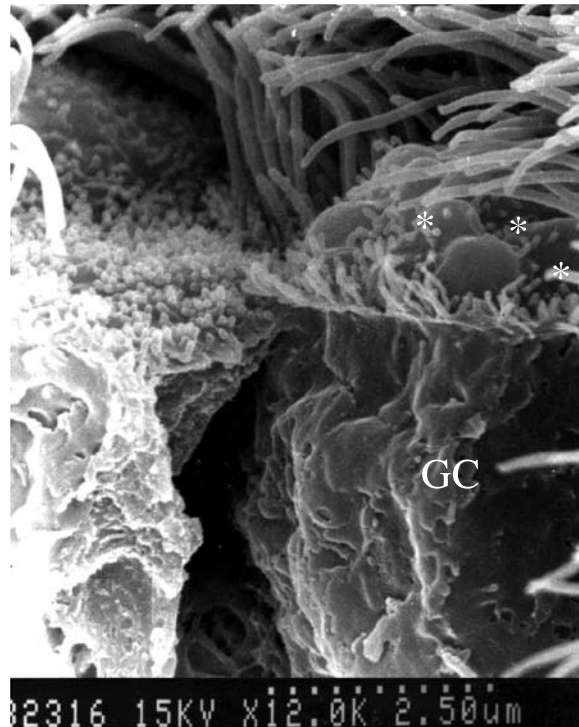


Figure 9 The lateral side of goblet cell (GC) was shown. The mucous granules (\*) were found on the top of the cell.

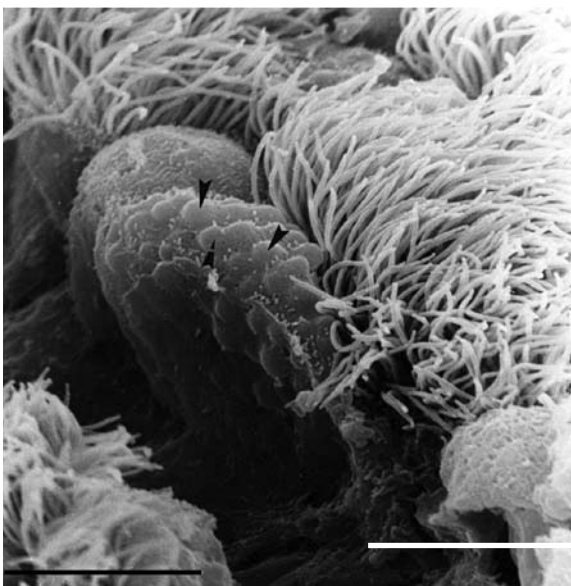


Figure 10 The numerous granules (arrowhead) in goblet cells were shown. X6000, bar = 5  $\mu$ m.

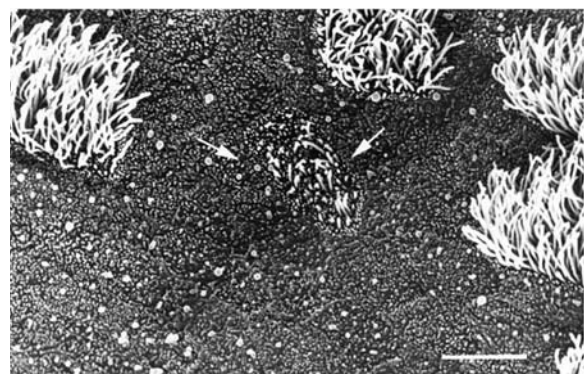


Figure 11 The young or immature cilia (arrows) were beginning to emerge. It was processing of ciliogenesis.

## Discussion

This present study finding the border line between trachea and primary bronchus was transitional zone. There was not different. It was prominent ciliated cell more than in the area of pharynx. Some ciliated cells, non-ciliated cell and goblet cells were lined by pseudostratified ciliated columnar epithelium refer as respiratory epithelium.

The proportion of ciliated cell and non-ciliated cell, in trachea were no significant different. The main function of ciliated cells is to propel the tracheobronchial secretions toward the pharynx. The mechanism of this process is called mucociliary escalator and well described<sup>5</sup>. The young or immature cilia were beginning to emerge. It was processing of ciliogenesis<sup>6</sup>. It has also been suggested that the microvilli surface important in regulating the volume of the tracheobronchial secretions, but whether this need in real remains to be determined<sup>7</sup>.

An important function of the membrane lining larger airways is the production of mucus, which covers the luminal surfaces. In general mammal, the mucous and serous were secreted by mucous and serous gland. From this observation, serous cells were not observed in the tracheal epithelial cells of Tupaia glis but mucous cells or goblet cells were obviously

observed. The mucus secretions were dominant releasing from the goblet cells. Goblet cells are abundant in the trachea of man, horse, sheep, guinea pig, cat and dog but relatively sparse in the rat, mouse, hamster and rabbit<sup>8,9</sup>. The mature mucus goblet cells have a characteristic goblet shape, caused by the contents of mucous granules. Their content is homogeneous, low density material. There was not significant different of the population of ciliated cells and non-ciliated cells in this mammal. The proportions of the population of ciliated and non-ciliated cells were varied from species to species<sup>10,11</sup>.

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