ONLINE FIRST

This is a provisional PDF only. Copyedited and fully formatted versión will be made available at final publication

HISTOLOGY AND HISTOPATHOLOGY

ISSN: 0213-3911
e-ISSN: 1699-5848

The multiple facets of the club cell in the pulmonary epithelium

Authors: López-Valdez Nelly, Rojas-Lemus Marcela, Bizarro-Nevares Patricia, González-Villalva Adriana, Casarrubias-Tabarez Brenda, Cervantes-Valencia María Eugenia, Ustarroz-Cano Martha, Morales-Ricardes Guadalupe, Mendoza-Martínez Shamir, Guerrero-Palomo Gabriela and Fortoul Teresa I

DOI: 10.14670/HH-18-713
Article type: REVIEW
Accepted: 2024-01-18
Epub ahead of print: 2024-01-18

The multiple facets of the club cell in the pulmonary epithelium

López-Valdez Nelly[1], Rojas-Lemus Marcela[1], Bizarro-Nevares Patricia[1], González-Villalva Adriana[1], Casarrubias-Tabarez Brenda[1], Cervantes-Valencia María Eugenia[1], Ustarroz-Cano Martha[1], Morales-Ricardes Guadalupe[1], Mendoza-Martínez Shamir[1], Guerrero-Palomo Gabriela[2], *Fortoul Teresa I[1].

1. Department of Cellular and Tisular Biology, School of Medicine, UNAM
2. Independent researcher.

Correspondence

Fortoul Teresa I. e-mail address: fortoul@unam.mx. Laboratorio de Morfología y Toxicología, Departamento de Biología Celular y Tisular, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM). Ciudad de México, 04510, México.

Acknowledgments

The authors thank Brenda Medina Rodríguez for tissue processing and Armando Zepeda-Rodríguez and Francisco Pasos-Nájera for the artwork in the figures, all from the Departamento de Biología Celular y Tisular, Facultad de Medicina, UNAM. The authors would like to thank MD Brenda Sandoval Meza, who is part of the Scientific Translation Department, Research Division at Facultad de Medicina, Universidad Nacional Autónoma de México, for translating this manuscript.

Funding

This research was partially supported by PAPIIT UNAM IN200418.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Abstract

The non-ciliated bronchiolar cell, also referred to as “club cell”, serves as a significant multifunctional component of the airway epithelium. While the club cell is a prominent epithelial type found in rodents, it is restricted to the bronchioles in humans. Despite these differences, the club cell's importance remains undisputed in both species due to its multifunctionality as a regulatory cell in lung inflammation and a stem cell in lung epithelial regeneration.

The objective of this review is to examine different aspects of club cell morphology and physiology in the lung epithelium, under both normal and pathological conditions, to provide a comprehensive understanding of its importance in the respiratory system.

Keywords: bronchiolar epithelium, metabolism, inflammation, club cell

Highlights

- The importance of non-ciliated bronchiolar epithelial cells or club cells in the lung is mainly related to inflammation regulation, epithelial regeneration, and detoxification.
- CC16, the main secretion product of club cells, regulates the inflammatory response of the lung.
- Club cells are critical in the development of specific pulmonary pathologies and further investigation is necessary to better understand the role of this cell and establish possible therapeutic applications.

Introduction

The mammalian respiratory system has a wide variety of epithelial cell types, such as: ciliated, secretory, basal, and neuroendocrine cells (Dean and Snelgrove, 2018). All of them play diverse and relevant roles in maintaining the homeostasis of the respiratory system (Schiller et al., 2019).

In recent decades, the non-ciliated bronchiolar cell (NCBC) or “club cell”, a type of secretory cell, has gained significant importance for its wide variety of functions. This cell, which is also known as "Clara's cell" after its discoverer, anatomist Max Clara, is a multifunctional cell located in the lung epithelium and participates in the metabolism of

xenobiotics, contributes to the regulation of the pulmonary immune system, and acts as a progenitor and regenerative cell, especially in response to damage of the bronchiolar epithelium (Reynolds and Malkinson 2010).

In human lungs, this cell is exclusively located within the epithelium of the distal conducting airways, specifically in the terminal bronchioles, representing approximately 80% of the epithelial population of the smallest bronchioles (Suarez et al., 2012). In rodents and other species, the club cell is present in the proximal airways such as the trachea (Boers et al., 1999). They also account for approximately 80% of the epithelial population in the terminal bronchioles.

This cell may play a significant role in the development of specific pulmonary pathologies, and the clinical aspects of its involvement are gradually becoming clearer (Rokicki et al., 2016).

History of club cell discovery

The first and original descriptions of bronchiolar cells were made in 1881 by Rudolph Albert von Kölliker, a Swiss physician, anatomist, and physiologist (Rokicki et al., 2016); however, his studies were not considered relevant at the time (Roma et al., 2021). Later, in 1928, Miller indicated that in the terminal bronchiolar lining, there were non-ciliated cells (Miller, 1928).

It was not until 1937 that the anatomist Max Clara took up this research and described a new type of secretory cell located in the human and rabbit bronchiolar epithelium, more than 50 years after Kölliker first identified these cells. This one was known as "Clara's cell" until 1955 (Winkelman and Noack, 2010).

Maximilian Josef Maria Clara, known as Max Clara, was born in a village near Bolzano in Italy-formerly Austria and now known as the province of South Tyrol-on February 12, 1899. He began his medical studies at the University of Innsbruck and later, in 1923, at the University of Leipzig, Germany. In his spare time, Clara devoted himself to studying histology. In 1935, he joined Nazi organizations such as the National Socialist German Workers' Party (NSDAP) and the German National Socialist Teachers' League (Donado et al., 2013).

Conveniently for many physicians who supported the Nazi party, in this same year, a petition was made to amend the law in order to allow the morphological study of prisoners' bodies from concentration camps, prisons, and interrogation centers. Naturally, this fact increased the number of bodies in the amphitheaters of German medical schools (Donado et al., 2013).

In March 1936, Max Clara benefited from the approval of the new law, and the results of his studies were based on the morphological analysis of lung tissue samples obtained from murdered prisoners around Dresden. Clara himself cited that his samples came from these bodies, preserved by vascular injection with fixative immediately after death (Winkelman and Noack, 2010). From his observations, Clara determined that the cell he had just described was specifically located in the epithelium lining the terminal and respiratory bronchioles and was morphologically characterized by secretory granules, a dome-shaped apical membrane, and no cilia (Donado et al., 2013).

In 1945, after the end of the war, Max Clara was arrested by the US government and released a year later. This fact made it impossible for him to obtain a job in his country, so he decided to immigrate to Turkey, where he was offered a position as a professor of Histology at the University of Istanbul. Clara remained there until 1961, finally returning to Munich, where he died in 1966 (Donado et al., 2013; Brenner et al., 2021).

In the present day, different authors have suggested changing the eponym of his cell due to the ethical controversies surrounding its discovery. Proposed changes to the term include non-ciliated bronchiolar cell or club cell, the latter being the most used due to its shape (Winkelman and Noack, 2010; Donado et al., 2013; Rokicki et al., 2016), and the one we will use in this text.

After Clara's descriptions, other researchers identified and described club cells in the lungs of other mammals. Macklin, for example, confirmed the presence of non-ciliated bronchiolar cells in the epithelium of the airways of mice, providing more information on their morphology, and Karrer first described the ultrastructure of these cells in mice in 1956 (Macklin, 1949; Karrer, 1956). For his part, Kisch was a pioneer in the description of the ultrastructure of club cells in rabbits (Kisch, 1958).

Morphological description of the club cell

As previously mentioned, Clara described the morphology of the club cell, which presents a cubic shape with a dome in the apical region that projects towards the bronchiolar lumen; it also has a basal nucleus and discrete cytoplasmic granules, which demonstrates its secretory function (Reynolds and Malkinson, 2010).

Ultrastructural analysis of this cell showed that, in its basal cytoplasm, there is a euchromatic nucleus with numerous grooves or ridges in the nuclear membrane. The Golgi apparatus and the rough endoplasmic reticulum, which have elongated cisternae and a limited number of ribosomes, are in proximity. There are many mitochondria with sparse ridges, especially in the apical region of the cell's cytoplasm; the prominent smooth endoplasmic reticulum (SER) is the predominant cytoplasmic constituent in many species like rodents and it is composed of short tubules. Also, the apical region contains cytoplasmic glycogen granules, approximately 10% of the cytoplasmic volume, and electron-dense spherical granules surrounded by a membrane that, according to morphometric analysis, measures approximately 0.3 μm in diameter (Rokicki et al., 2016; Chookliang et al., 2021). Figure 1 summarizes the morphological characteristics of club cells.

It is important to highlight that club cells have been identified in many mammalian species, however, some ultrastructural characteristics of this cell are consistently present in most species, such as the basal nucleus location and lateral cytoplasmic extensions.

On the other hand, there are relevant differences concerning some organelles, such as the well-developed SER that occupies most of the apical cell cytoplasm that is present in almost all species, however, in primates and carnivores, this feature does not seem to be so relevant.

Another difference is the presence of glycogen, which is rarely observed in most species. The following table summarizes the relative content (expressed as a percentage) of the most important organelles of club cells in different species, including rodents, carnivores, primates, and lagomorphs, among others.

It is important to mention that the ultrastructural characteristics correspond to the characterization of club cells in the distal airways (mainly bronchioles) of different species. In addition to interspecific morphological differences, there are also differences at different levels of the airways within the same species, across the regions of the airways. In humans, for example, Basset and Cols described non-ciliated bronchiolar cells in the proximal airways as cylindrical with dense homogeneous electron-dense ovoid granules, while in the distal airways; the cells are cubic as described above, with more ovoid heterogeneous granules (Basset et al., 1971). Plopper and Cols also reported differences in the case of rabbits; in the proximal airways, such as the trachea, club cells are columnar, with an ovoid basal nucleus surrounded by tubules of rough endoplasmic reticulum, the apical cytoplasm contains dense ovoid granules, SER, and long regular microvilli on the apical edge. On the other hand, in distal airways, the cells are cuboid, with a basal nucleus with irregular edges, an apical cytoplasm full of ovoid electron-dense granules, a well-developed SER, and abundant mitochondria (Plopper et al., 1983).

Another important aspect to consider is the fact that club cell morphology might be altered under different conditions and thus modify its function. For example, in diabetic rats, it was reported that the cells had pyknotic nuclei. At the same time, an ultrastructural analysis identified characteristics such as an increment in nucleoplasm heterochromatin-mainly in the nuclear periphery-hypertrophic mitochondria with damaged crests and membranes, electron-lucid secretory granules, and desquamation of these cells towards the bronchiolar lumen (Chookliang et al., 2021). Conversely, the exposure of mice to toxic agents such as naphthalene increased the size of club cells due to the formation of vacuoles in their cytoplasm (Carratt et al., 2019).

Club cells present the classic morphology associated with their secretory activity. However, it is worth mentioning that it presents some other prominent organelles, such as SER, which is related to its lung xenobiotic metabolism, as well as other functions that will be detailed throughout the text.

Functions of club cells in the lung

Lung epithelial regeneration

As previously mentioned, the club cell has multiple functions within the lung epithelium. One of its most interesting and recently explored functions is the role it plays as a stem cell of the bronchiolar epithelium.

Like most cells in the airway epithelium, the club cell is derived from the endoderm during embryonic development. Club cell pools that are present and proliferate throughout postnatal life are established at this stage of prenatal life (Reynolds and Malkinson, 2010) and are especially abundant in certain regions. For example, in the normal lung, these cells could represent 15% of the population in the terminal bronchioles and 44% in the respiratory bronchioles of proliferating airway epithelial cells (Boers et al., 1999).

Interestingly, club cells maintain their ability to redefine their phenotype in response to alterations in the lung caused by microenvironmental influences or exposure to environmental agents (Reynolds and Malkinson, 2010). An example of this is the change of the cell to a mucosecretory phenotype, a phenomenon known as mucoid metaplasia that is observed after exposure to various substances, such as ozone and vanadium (López-Valdez, 2020). Figure 2 illustrates mucoid metaplasia.

In a normal lung, club cell proliferation maintains facultative progenitor populations and restores populations of terminally differentiated cells such as secretory (Stripp et al., 1995) and ciliated (Rawlins et al., 2009). However, when the club cell population is reduced due to different factors, such as exposure to specific cytotoxic compounds like naphthalene, a bronchiolar tissue-specific stem cell or vCE is activated. This cell type is in the neuroepithelial bodies and at the bronchioloalveolar junctions (Stripp and Reynolds, 2008). Although this cell is rare and confined to specific microenvironments, it can initiate and sustain an epithelial repopulation in the terminal bronchioles (Reynolds et al., 2000). Other cellular characteristics include a slow cell cycle; it is relatively undifferentiated as it does not express CYP2F2, although it expresses Clara cell protein (CCSP) or CC16. These last characteristics correspond to mature CC (Reynolds and Malkinson, 2010). The naphthalene damage model to the bronchiolar epithelium is widely used to explore the

mechanisms of club cell replacement and airway regeneration in experimental mice (El Agha and Thannicka, 2023).

It is relevant to mention that under conditions of homeostasis, club cells of proximal airways like the trachea, are not the principal progenitor cells of the epithelium, and their potential is limited to originating ciliated cells (Rawlins et al., 2009). In the case of the distal airways, evidence suggests that the club cell is the principal progenitor, both in conditions of homeostasis and damage (Reynolds and Malkinson, 2010).

Lineage tracing studies have been one of the most relevant elements in club cell identification as progenitors in the lung. There is now *in vivo* evidence in mice suggesting the differentiation of Scgb1a1+ (CC16+) cells into type I and type II pneumocytes following damage from bleomycin exposure or influenza infection in areas close to bronchoalveolar ducts (Zheng et al., 2012). These cells were originally described by Kim and Cols as a lineage that they named bronchoalveolar stem cells or BACs (Scgb1a1+, Sftpc+), located at the junction of the bronchoalveolar duct in mice (Kim et al., 2005; Lee et al., 2013). Salwig and Cols demonstrated that they are essential in the recovery of the epithelium after the generation of some types of damage (Salwig et al., 2019).

In humans, it has been proposed that “respiratory airway secretory cells” or RASCs positive for the marker Scgb3a2, which has been localized in some subpopulations of club cells in the more distal airways, could represent a functional equivalent of BACs (Yang et al., 2017; Basil et al., 2022). Other interesting *in vitro* data have shown that club cells are capable of dedifferentiating into basal cells that proliferate and differentiate mature cells such as ciliated, secretory, and basal cells (Tata et al., 2013).

It is important to clarify that the well-known type II pneumocyte is responsible for the regeneration of the alveolus because it originates the type I pneumocyte (Rawlins et al., 2009).

The figure 3 resumes the origin and plasticity of club cells.

Metabolism of xenobiotics

One of the club cell's best-known functions is metabolizing xenobiotic agents because the cell is located at the main site in the lung epithelium where proteins of the cytochrome P450 (CYP450) enzyme system are expressed. Also, mixed-function monooxygenase elements are expressed and are responsible for the metabolic activation of inhaled therapeutic agents and xenobiotics, converting them into intermediates with pharmacological or toxicological effects, as the case may be (Aoshiba et al., 2014).

Through respiration, humans are exposed to multiple xenobiotics that can cause damage to the respiratory tract, specifically to club cells that become targets of these xenobiotic compounds when expressing metabolic proteins.

Naphthalene is one of the xenobiotics that is released into the atmosphere through emissions generated by tobacco smoke and fossil combustion, and its effect on club cells has been extensively studied (Aoshiba et al., 2014). Various experimental models have identified that acute exposure to this compound, either intraperitoneally or by inhalation, causes vacuolization and swelling in club cells, as well as cellular exfoliation in the lumen of the respiratory tract leading to massive loss of club cells by necrosis. These effects are related to the bioactivation of naphthalene by CYP2F2, converting it into cytotoxic secondary metabolites. This generates extensive injury to the airway epithelial zones, which are repaired by naphthalene-resistant club cells and tissue stem cells associated with tracheobronchial glands, neuroepithelial bodies, and the junction of bronchoalveolar ducts (Aoshiba et al., 2014; Carratt et al., 2019).

It is important to mention that CYP2F2 is one of the most expressed cytochromes in club cells in mice. In contrast, this cell in humans expresses an orthologous equivalent known as CYP2F1, which is also involved in the biotransformation of xenobiotics (Cohen et al., 2020). Additionally, there is evidence that exposure of this epithelial cell to other environmental pollutants, such as dioxins and polycyclic aromatic hydrocarbons, has been associated with respiratory diseases such as asthma, chronic obstructive disease, and lung cancer. This fact has been related to the presence of CYP1A1 and CYP1B1, enzymes that bioactivate these xenobiotics in humans and animal models (Chang et al., 2006; Liu et al.,2021; Wang et al., 2021;).

Furan is another compound that, besides being present in the environment, has also been detected in food and beverages and has been reported to induce liver and lung damage. Although evidence regarding its toxicity in club cells is not extensive, it is known that the club cell is a target of the effects of this compound, probably due to its bioactivation by CYP2E1 and conversion into the more reactive and toxic metabolite known as cis-2-butene-1,4-diol (Tăbăran et al., 2019).

The involvement of club cells in the metabolism of many of the xenobiotics to which humans are exposed, mainly through inhalation, is conclusive, as is the role of the enzymes in activating and detoxifying these substances. These characteristics make club cells vulnerable to the effects of these compounds, making them part of developing various pulmonary pathologies associated with exposure to xenobiotics.

The role of club cells in the regulation of inflammation in the lung

Another outstanding function of club cells is their capacity to regulate the pulmonary inflammatory response in different conditions. For example, there is evidence that patients diagnosed with asthma or chronic lung diseases have lower quantities of this cell and some products secreted by them, indicating that this cell plays a critical role in the pathogenesis of these conditions (Almuntashiri et al., 2020).

Experimental evidence suggests that club cells synthesize and secrete different factors that can be immunomodulatory, including the secretory protein CCSP or CC16, which is also its main secretion product. Other molecules with this function have also been identified but have yet to be studied (Singh and Katyal, 2000; Kimura et al., 2022).

Initially, it was shown that club cells could decrease the inflammatory response through their effect on macrophages. In this regard, Snyder et al. demonstrated that genetically modified mice deficient in club cells or CC16 showed an intense inflammatory response because macrophages showed high TLR4 expression and increased production of TNFα after being exposed to lipopolysaccharides (LPS), compared with wild-type mice. However, when CC16 was added to macrophages cultured with LPS, there was no attenuation of TNFα production, suggesting that the response may be mediated by other proteins in the cell that have not yet been identified. Therefore, further studies are needed (Snyder et al., 2010).

Also, in a murine model of allergic rhinitis, it was reported that the club cell has anti-allergic effects because of CC16, and inhibits the response of TH17 cells since it regulates the function of dendritic cells. This fact is interesting as the TH17-mediated immune response stimulates eosinophil recruitment in allergic airway diseases, stimulated by the TH2 response (Liu et al., 2013).

CC16 has several functions in modulating the immune response in the lung, for example, the synthesis of cytokines such as neutrophils, macrophages, and lymphocytes, which regulate the recruitment and activity of immune system cells. It also has a prominent role in the immune organization against pathogens such as bacteria and fungi and is involved in the production of immunoglobulins, such as pulmonary and serum IgA. In addition, experimental and clinical evidence indicates that CC16 is a negative lung inflammation regulator.

One of the first mechanisms of the anti-inflammatory action of CC16 described was the inhibition of phospholipase A2 (PLA2) involved in the production of proinflammatory lipid mediators to promote neutrophil recruitment (Anderson et al., 1994). In accordance with these findings, it was reported that CC16 deficiency *in vitro* (Dudek et al., 2011) and *in vivo* (Yoshikawa et al., 2005) induces elevated PLA2 activity and considerable neutrophil recruitment.

On the other hand, *in vivo* studies support the role of CC16 in the regulation of cytokine production: it has been shown that mice that have deficient production of this protein present an increased inflammatory response when sensitized with ovalbumin, and express higher levels of pro-inflammatory cytokines such as IL-4, IL-5, IL-9, and IL1-13 (Chen et al., 2001).

Also, it was shown that CC16 can regulate the Th2-type response in the lung. In a model of lung allergy in mice, it was demonstrated that when the CC16 gene is absent, there are elevated levels of Th2 response cytokines. However, there are no changes in the number of T cells because there is no cell proliferation. This fact suggests a modulatory effect on cytokine secretion by lymphocytes. The significant reduction in the gene expression of interleukins includes IL-4, IL-5, and IL-13, which are part of the Th2 response (Hung et al.,

2004). Furthermore, Liu and Cols identified in their murine allergic rhinitis model, where mice presented a decrease in CC16, that exogenously administering this protein decreased the cytokines of the Th2 response and the number of Th17 cells (Liu et al., 2013).

In vitro, CC16 has been shown to attenuate inflammation by decreasing the production of IL-8 (a pro-inflammatory cytokine) in bronchial cells, which contributes to decreasing the infiltration of cells such as neutrophils (Tokita et al., 2014). Other *in vitro* evidence has shown that CC16 is capable of directly binding to IL-8, inhibiting neutrophil chemotaxis, and reversing the migratory behavior of these cells promoted by cigarette smoke (Knabe et al., 2019). On the other hand, in a murine model of *Mycoplasma pneumonia*, it was reported that CC16 can bind to the α4β1 receptor, also known as the adhesion molecule VLA-4 that is expressed in the endothelium of pulmonary blood vessels, interfering with the recruitment of leukocytes (Johnson et al. al., 2021).

Another mechanism that explains the immunomodulatory activity of CC16 is the inhibition of NF-κβ factor activation, which is achieved by preventing phosphorylation of its receptor, leading to a decrease in inflammation (Hiemstra and Bourdin, 2014).

The club cell is also involved in producing immunoglobulins and in the organization of mucosa-associated lymphoid tissue in the lung. In mice lacking the gene encoding the CC16 protein, an increase in the number of peribronchiolar lymphocytes expressing IgA was detected; there was also evidence of an increase in IgA in both mRNA and protein levels in the lung, as well as a significant increase in serum IgA levels (Watson et al., 2001).

On the other hand, when there is chronic exposure to different pollutants, generally there is a decrease in the expression of CC16 in the bronchiolar epithelium, and organisms become more susceptible to developing an inflammatory response in the lung. For example, Gowdy et al. in a murine model of diesel-derived particulate matter (DEPs) inhalation, reported that the decrease in CC16 and surfactant proteins (SP-A and SP-D) in lung homogenates and bronchoalveolar lavage coincides with the over-expression of pro-inflammatory cytokines such as IL-6, IL-13, and TNF-α (Gowdy et al., 2008).

It is interesting to mention that, in other studies, the tendency is toward increased CC16 because of xenobiotic exposure. López-Valdez and coworkers reported that the inhalation of vanadium increased the expression of CC16 in mouse club cells (López-Valdez, 2020). Other studies reported the involvement of CC16 in promoting bronchiolar epithelial remodeling in a model of ovalbumin exposure in models of allergic inflammation (Wong et al., 2009).

These pieces of evidence show the importance of this protein in the modulation of acute and chronic inflammation in the lung and explain why the decrease or absence of this protein contributes to the development of different pulmonary pathologies.

There is also evidence that demonstrates the inhibitory action of inflammation on CC16 production. In this regard, a negative correlation was demonstrated between local eosinophilia and the presence of pro-inflammatory markers and the production of CC16 in the nasal mucosa of patients with allergic rhinitis, in whom CC16 levels in the nasal fluid were lower than in control individuals (Johansson et al., 2005; Benson et al., 2007; Perić et al., 2017). The above suggests that the decrease in CC16 could contribute to the development of allergic inflammation.

Based on other compelling data, the club cell is been found to have a range of functions, including the regulation of circadian rhythms in the lung and immunomodulation. For example, the nuclear REV/ERB receptors located on club cells have been found to have an anti-inflammatory role; and *Bmal1* genes-central elements in the circadian clock-are expressed in these cells. Their deletion has been associated with exaggerated inflammatory responses due to allergenic stimuli and microorganisms (Ince et al., 2018).

Moreover, pro-inflammatory effects mediated by club cells have been reported. For example, there is a study showing that the allergenic fungal protease Alp-1 (aeroallergen alkaline protease from Aspergillus sp) is inhaled and disrupts intercellular junctions between club cells. They detect the damage through the transmembrane sensor TRPV4, coupled with a calcium channel, and allergic inflammation is promoted through calcineurin signaling. In addition, the authors dispute evidence that people with asthma have an increased expression of the TRPV4 sensor in club cells (Weisner et al., 2020).

Murine experimental models have shown that the club cell stimulates the production of pro-inflammatory cytokines in the lung in response to intratracheal treatment with LPS, which mediates the innate immune response of the lung. Keratinocyte-derived chemokine (KC) was expressed in non-ciliated bronchiolar cells, type II pneumocytes, and alveolar macrophages, and transforming growth factor α (TNF-α) was expressed in alveolar macrophages. In addition, KC has a chemotactic function for neutrophils, and TNF-α participates in inflammatory responses (Elizur et al., 2007).

In another murine model of fungal pneumonia with *Blastomyces dermatitidis*, activation of the nuclear factor kB (NF-kB) signaling pathway in pulmonary epithelial cells was reported, which is essential in the development of innate antifungal immunity. This study also identified the upregulation of cytokines involved in the antifungal response, such as interleukin 17A (IL-17A) and granulocyte-monocyte colony-stimulating factor (GM-CSF). Activation of the NF-kB pathway increases the TCRb+CD4+ (nTh17) pool, the innate GM-CSF-producing lymphocytes. Lung epithelial cells also produce interleukin-1^{a} (IL-1^{a}), which is involved in the proliferation of nTh17 lymphocytes, thus favoring the immune response against the fungus[29] (Hernández-Santos et al., 2018). Club cells also produce IL-17 in immunity against bacteria in the lung, e.g., *Klebsiella pneumoniae*. In addition, IL-17 secretion by these cells stimulates the secretion of chemokines (CXCL5), leading to neutrophil recruitment (Chen et al., 2016).

Different data suggest the involvement of other mechanisms associated with club cell-regulated pro-inflammation, such as senescence. Ongoing damage to the airways has been identified to increase the p53 protein in club cells, which induces senescence. This condition has been associated with increased chronic inflammation and COPD (chronic obstructive pulmonary disease)-like features. These findings were observed in mice that underwent repeated exposure to LPS, resulting in the development of severe chronic bronchitis. The mice were then compared to those with club cells genetically modified to have no p53 expression. The genetically modified mice showed surprisingly less inflammation and lung damage. In this same experiment, senescent cells were eliminated pharmacologically by Bcl-2 inhibitors, and a protective effect was also observed: less inflammation and lung damage (Sagiv et al., 2018)

All findings presented in this section demonstrate the crucial role club cells play in the regulation of the pulmonary immune response, either pro- or anti-inflammatory. This fact has relevant implications both in physiological conditions-where club cells would moderate inflammatory responses-and in chronic diseases, where the modified number and function of the cells would alter the regulation of this inflammatory response.

Clara Cell Secretory Protein (CCSP or CC10 or CC16)

As previously mentioned, CC16 is the primary secretory product of club cells and one of the most abundant proteins in the lung (Martinu et al., 2023). Different names for this protein have been reported in the literature, such as CCSP (Clara cell secretory protein), CC10 (Clara cell secretory 10-kDa protein, 10k-Da Clara cell protein), CC16 (Clara cell secretory 16-kDa protein, 16k-Da Clara cell protein), human protein 1, urine protein 1, PCB-BPs (polychlorinated biphenyl binding protein) and secretoglobin SCGB1A1 (secretoglobin family 1A member 1) (Anderson et al., 1994).

CCSP or CC16 is a protein mainly expressed in the respiratory tract and it is synthesized and constitutively secreted by club cells in mammals (Martinu et al., 2023), as shown in Figure 4.

CC16 is like the uteroglobin found in rabbit endometrium during early pregnancy (Singh et al., 1990; Bernard et al., 1993) and is expressed in low amounts in extrapulmonary tissues such as the thymus, prostate, pituitary, and bone marrow (Martinu et al., 2023).

It is relevant to mention that, in the past, it was thought that CC16 was a specific marker of airway club cells; however, it is now known through the application of molecular biology techniques that it is also expressed by other secretory cells of the respiratory epithelium of the upper airways that belong to subpopulations of mucosecretory and serous cells (Hay et al., 1995).

CC16 is secreted into the airway lumen and can be detected in bronchoalveolar lavage (BAL) at a concentration of 1,000-2,000 µg/L, while in serum, a concentration of 10-15 µg/L is reported in healthy individuals. CC16 has also been isolated as a peripheral pulmonary marker to evaluate the cellular integrity or permeability of the pulmonary epithelium since the protein increases its concentration in serum when the permeability of

the epithelium is altered. This protein can also be detected in pleural fluid, sputum, urine, cerebrospinal fluid, and amniotic fluid, evidencing its importance as a marker of club cell development or damage (Bernard et al., 1993; Bernard et al., 1994; Laucho et al., 2016;).

CC16 is a homodimeric protein with a low molecular weight. Depending on the species, it consists of two chains composed of 70 to 76 amino acids (approximately 16 kDa) which form four α helices separated by β-hairpins, joined through two disulfide bridges formed between Cis^{3} and $Cis^{69'}$. Together, the eight α helices form a hydrophobic pocket where the protein stores phosphatidylcholine and phosphatidylinositol, the main components of pulmonary surfactant (Umland et al., 1994). In humans, the molecular mass of this protein is exactly 15.84 kDa (Bernard et al., 1993), while in rodents, like rats and mice, CC16 has a molecular weight of 16-17 kDa (Halatek et al., 1998). It is relevant to mention that the abbreviation CC10 given to Clara cell protein is based on an underestimation of the size of the protein by SDS-PAGE (Bernard et al., 1993).

The relevance of this protein is mainly related to its immunomodulatory role, which was explained previously. As mentioned, it is also considered an important marker of lung epithelial damage.

Alteration of CCSP secretion in different pathological conditions and its role as a marker of damage

For years, the role of CC16 concerning respiratory diseases has been investigated and approached from different perspectives. The quantification of this protein in serum, plasma, sputum, BAL, nasal lavage fluid, tracheal aspirates, and urine has contributed to explaining its relevance in lung pathology as well as its use as an essential marker of bronchiolar epithelial damage (Martinu et al., 2023).

Studies in experimental models in CC16-/- (CCSP-/-) mice have shown that the absence of this protein results in changes in club cell structure, such as decreased cell number, fewer secretory granules, decreased RER and changes in the Golgi apparatus, increased susceptibility to epithelial damage from exposure to contaminants, as well as increased susceptibility to oxidative stress damage, presenting increased production of pro-inflammatory cytokines and decreased CYP2F2 cytochrome concentrations, data suggesting a protective role in cases of oxidative stress (Wong et al., 2009).

In general, the evidence indicates that acute exposure to factors that induce bronchiolar epithelial damage leads to increased plasma CC16 levels due to increased lung permeability (Martinu et al., 2023). Intratracheal administration of LPS at high concentrations decreases the number of club cells in rat bronchiolar epithelium, which increases serum CC16 protein, suggesting increased permeability of the bronchiolar epithelium (Arsalane et al., 2000). In humans, CC16 elevation has also been reported in cases of pulmonary fibrosis (Buendia et al., 2016) and sarcoidosis (Hermans et al., 2001).

In chronic exposures, there is a loss or decrease in club cells that lead to CC16 deficiency in BAL and serum; such is the case of exposure to cigarette smoke, pollutants, and occupational exposures, among others (Zhai et al., 2018; Beamer et al., 2019; Arsalane et al., 2000; Martinu et al., 2023).

In the case of respiratory infections, the presence of CC16 varies. In children with Respiratory Syncytial Virus infection, the protein was elevated in serum compared with controls, while in the case of parainfluenza virus infection, no differences were reported. In infants under one year of age diagnosed with bronchiolitis, an increase in urine protein was reported, and this finding correlated with the severity of bronchiolitis. In the case of asthma, a decrease in serum protein concentrations was observed, although other studies reported no differences (Almuntashiri et al., 2020).

Voraphani et al. reported a decrease in circulating CC16 concentrations in cases of impaired lung function, and that this decrease could be a predictor of further FEV1 loss during childhood and of accelerated lung function deficiency in adults in the general adult population (Voraphani et al., 2023). Using three sources of information from population-based birth cohorts, they found an inverse relationship between active asthma and low CC16 in serum. If asthmatics were classified as having frequent or infrequent symptoms, CC16 was lower in the first group. When circulating CC16 was measured in all the cohorts of asthmatics from childhood to young adulthood, with normal lung function and frequent symptoms, CC16 was low in a dose-response relationship. With their findings, they propose CC16 as a possible biomarker for predicting clinical course in children with asthma (Voraphani et al., 2023).

In subjects with COPD, a decrease in CC16 in serum and airway bronchial lavage was also evidenced and CC16 is suggested as a possible biomarker of illness severity and progression (Park et al., 2013; Guerra et al., 2015). Zhai et al. suggested that, at least in mice, CC16 could be part of the reduction in lung function because of the increased airway remodeling observed in low-CC16 mice (Zhai et al., 2019).

Not only CC16 has been analyzed in asthmatic patients. A report by Buendía-Roldán et al., in patients with interstitial lung fibrosis, measured CC16 in serum and BAL and identified its immuno-colocalization in tissue sections, observing an increase in CC16 compared with controls (Buendia-Roldán et al., 2016).

A case-control study by Zhain et al. in children with cystic fibrosis found that a decrease in CC16 was strongly associated with the severity of decreased lung function and systemic inflammation (Zhai et al., 2022). Also, a reduction in serum and BAL CC16 in obese patients suggested the relevant participation of CC16 in the development and severity of asthma in this population. Furthermore, Rohmann et al. propose that CC16 is not only affected by pulmonary illness but also by metabolic and cardiovascular ailments, which suggest a more complex regulation of serum CC16 concentrations that needs further analysis (Rohmann et al., 2023)

It should be noted that some factors could increase the expression of CC16, which is relevant from a therapeutic point of view in the case of pathologies related to the decrease in this protein (Martinu et al., 2023). Concerning this point, it has been reported that retinoids *in vitro* and oral administration of vitamin A *in vivo* increase CC16 levels in serum (Chen et al., 2017). Other studies indicated that glucocorticoids *in vitro* increase CC16 expression (Berg et al., 2002). In *in vivo* cigarette smoke inhalation models, data indicate that the administration of roflumilast (phosphodiesterase 4 inhibitor) can reverse the decrease in CC16 in the airways (Ge et al., 2009).

Regarding pulmonary diseases and their relationship with CC16, more knowledge still needs to be revealed about the role of the protein in each of these entities, and further studies are required to understand its functions and possible therapeutic applications.

In general, it is known that in obstructive pulmonary diseases such as COPD (Park et al., 2013; Guerra et al., 2015; Milne et al., 2020), asthma (Guerra et al., 2015; Voraphani et al., 2023), and cystic fibrosis (Zhai et al., 2022), CC16 protein levels decrease. In contrast, in restrictive diseases such as idiopathic pulmonary fibrosis (Buendia et al., 2016), emphysema (Kokuho et al., 2015), and Acute Respiratory Distress Syndrome (ARDS) (Lin et al., 2018), CC16 protein levels increase. In patients with SARS-CoV-2, elevated levels of CC16 on the day the patient was admitted to hospital were associated with a four-fold risk of developing ARDS with a greater risk of a fatal outcome (Tiezzi et al., 2021).

Finally, club cells and CC16 are associated with the pathogenesis of chronic lung allograft dysfunction (CLAD), identified by an irreversible decline in lung function, which represents the most common cause of late death after lung transplantation (Verleden et al., 2019). CLAD is associated with the reduction in CC16 in BAL (Nord et al., 2002; Itabashi et al., 2021) and serum (Nord et al., 2002). Bronchiolitis obliterans syndrome (BOS) is the most common CLAD manifestation and is characterized by airway obstruction and histological findings of bronchiolitis obliterans (BO). BO is related to decreased numbers of CC16-expressing epithelial cells suggesting a reduction in the actual number of club cells (Kelly et al., 2012) and club cell ablation causes BO in a mouse model of lung transplantation (Liu et al., 2019). This evidence suggests that club cells and CC16 appear to be protective against CLAD and BO, however further studies are needed to confirm this.

CBNC Club cells and regulation of the pulmonary circadian cycle

A recently investigated phenomenon is the role of the club cell in regulating the pulmonary circadian cycle. In mammals, the central regulator of circadian cycles is the suprachiasmatic nucleus located in the hypothalamus; however, it has been identified that, alternatively, organs such as the lung possess their own circadian clock. Some of the mechanisms associated with this are related to the response of lung cells to different environmental stimuli.

In the mouse lung, the club cell has been found to express the *CLOCK* and *PER2* genes, *CLOCK* being one of the central genes of the biological clock. The study executed by Gibbs et al. demonstrated the co-expression of CLOCK and PER2 gene proteins in club cells and type II pneumocytes and identified that the loss of club cells in the bronchiolar

epithelium caused by naphthalene treatment significantly altered the rhythmicity of the lung clock (Gibbs et al., 2009).

The same group demonstrated that deletion of the *Bmal1* gene in mouse lung club cells alters the expression of other circadian cycle regulatory genes, such as the *REV-ERB-α* gene (Gibbs et al., 2014). This evidence shows the important role of these epithelial cells as regulators of lung rhythmicity.

Interestingly, other studies show that circadian expression of biological clock genes in club cells regulates acute inflammation in the lung through cytokine release and neutrophil recruitment (Pariollaud et al., 2018).

Conclusions

The importance of non-ciliated bronchiolar epithelial cells or club cells is related to multiple functions within the lung epithelium such as CC16 secretion, epithelial regeneration, detoxification, inflammation regulation, and regulation of lung timing (Fig. 5).

The relevance of club cells in the pulmonary epithelium becomes evident upon analysis of the diverse functions they perform in the lung. Their role in regulating lung inflammation and their association with various pathological conditions are particularly noteworthy; however, further investigation is necessary to better understand the role of this cell and thus establish possible therapeutic applications.

Figures

Figure 1. Representation of the ultrastructural appearance of the club cell. This image was created with Biorender.com.

Figure 2. Mucoid metaplasia in mouse bronchiolar epithelium in a model of vanadium exposure. After vanadium inhalation, the presence of magenta-labeled PAS-positive cells in the bronchiolar epithelium is observed. PAS stain. Scale bar: 50 μm. The photomicrograph is an image from original research by the authors.

Figure 3. Club cell origin and plasticity: In a normal lung, club cells origin from basal cells. Proliferation maintains facultative progenitor populations of club cells and restores populations of terminally differentiated cells such as secretory. This image was and created with Biorender.com.

Figure 4. Club cell CC16 detection by immunohistochemistry. The ochre color demonstrates the positive mark of the protein in mice bronchiolar epithelium. Immunohistochemistry. Scale bar: 50 μm. The photomicrograph is an image from original research by the authors.

Figure 5. Club cell functions summary. This image was created with Biorender.com.

Table 1. Relative content of cellular components of club cells in different species. Modified from Plopper y Hyde, 2015. Epithelial Cells of the Bronchiole.

Species	Nucleus	Lateral cytoplasmic extensions	Smooth Endoplasmic Reticulum	Secretory granules	Glycogen granules	Mitochondria	References
Rat	29%	+	66%	+	0.1%	16%	Young et al., 1986; Plopper et al., 1994
Mouse	22%	+	55%	+	0	35%	Pack et al., 1980, 1981
Hamster	25%	+	79%	+	0	11%	Plopper et al., 1980a,
Rabbit	24%	+	62%	+	7%	19%	Plopper et al., 1980a; Hyde et al., 1983
Dog	23%	+	25%	+	57%	8%	Plopper et al., 1980b
Cat	27%	+	11%	-	61%	20%	Plopper et al., 1980b, Hyde et al., 1985
Macaque	29%	+	0.5%	+	0%	14%	Tyler and Plopper 1985; Plopper et al., 1989
Sheep	26%	+	65%	+	7%	14%	Plopper et al., 1980b; Mariassy and Plopper 1983
Cow	28%	+	22%	+	62%	12%	Mariassy et al., 1975; Plopper et al., 1980b
Human	42%	+	0.3%	+	5%	15%	Plopper et al., 1980a; Shijubo et al., 1997, 1999; Boers et al., 1999
+ = present, - = absent							

References

Almuntashiri S., Zhu Y., Han Y., Wang X., Somanath P.R. and Zhang D. (2020). Club cell secreted protein CC16: potential applications in prognosis and therapy for pulmonary diseases. J. Clin. Med., 9, 4039.

Andersson O., Nordlund-Möller L., Barnes H.J. and Lund J. (1994). Heterologous expression of human uteroglobin/polychlorinated biphenyl-binding protein. Determination of ligand binding parameters and mechanism of phospholipase A2 inhibition in vitro. J. Biol. Chem. *269*, 19081-19087.

Aoshiba K., Tsuji T., Itoh M., Semba S., Yamaguchi K., Nakamura H. and Watanabe H. (2014). A murine model of airway fibrosis induced by repeated naphthalene exposure. Exp. Toxicol. Pathol. 66, 169-177.

Arsalane K., Broeckaert f., Knoops B., Wiedig M., Toubeau G. and Bernard A. (2000). Clara cell specific protein (CC16) expression after acute lung inflammation induced by intratracheal lipopolysaccharide administration. Am. J. Resp. Crit. Care Med., *161*, 1624-1630.

Basil M.C., Cardenas-Diaz F.L., Kathiriya J.J., Morley M.P., Carl J., Brumwell A.N., Katzen J., Slovik K., Babu A., Zhou S., Kremp M., McCauley K.B., Li S., Planer J.D, Hussain S.S., Liu X., Windmueller R., Ying Y., Stewart K.M., Oyster M., Christie J.D., Diamond J., Engelhardt J.F., Cantu E., Rowe S.M., Kotton D.N., Chapman H.A. and Morrisey E.E. (2022). Human distal airways contain a multipotent secretory cell that can regenerate alveoli. Nature 604, 120-126.

Basset F., Poirier J., Le Crom M. and Turiaf J. (1971). Ultrastructural study of the human bronchiolar epithelium. Z. Zellforsch. Mikrosk. Anat. 116, 425-442.

Beamer P.I., Furlong M., Lothrop N., Guerra S., Billheimer D., Stern D.A., Zhai J., Halonen M., Wright A.L. and Martinez F.D. (2019). CC16 levels into adult life are associated with nitrogen dioxide exposure at birth. Am. J. Resp. Crit. Care Med. 200, 600-607.

Benson M., Fransson M., Martinsson T., Naluai Å.T., Uddman R. and Cardell L.O. (2007). Inverse relation between nasal fluid Clara Cell Protein 16 levels and symptoms and signs of rhinitis in allergen-challenged patients with intermittent allergic rhinitis. Allergy 62, 178-183.

Berg T., Cassel T.N., Schwarze P.E. and Nord M. (2002). Glucocorticoids regulate the CCSP and CYP2B1 promoters via C/EBPβ and δ in lung cells. Biochem. Biophys. Res. Commun. 293, 907-912.

Bernard A., Marchandise F.X., Depelchin S., Lauwerys R. and Sibille Y. (1992). Clara cell protein in serum and bronchoalveolar lavage. Eur. Resp. J. *5*, 1231-1238.

Bernard A., Dumont X., Roels H., Lauwerys R., Dierynck I., De Ley M., Stroobant V. and de Hoffmann E. (1993). The Molecular-mass and Concentrations of Protein-1 Or Clara Cell Protein in Biological-fluids-a Reappraisal. Clin. Chim. Acta, *223*, 189-191.

Bernard A., Thielemans N., Lauwerys R., Langhendries J.P., Lierde M.V. and Freund M.M. (1994). Clara cell protein in human amniotic fluid: a potential marker of fetal lung growth. Pediatr. Res. 36, 771-775.

Boers J.E., Ambergen A.W. and Thunnissen F.B. (1999). Number and proliferation of clara cells in normal human airway epithelium. Am. J. Respir. Crit. Care Med. 159, 1585-1591.

Brenner E., De Caro R. and Lechner C. (2021). Max Clara and Innsbruck - The origin of a German Nationalist and National Socialist career. Ann. Anat. 234, 151662.

Buendía-Roldán I., Ruiz V., Sierra P., Montes E., Ramírez R., Vega A., Salgado A., Vargas M.H., Mejía M., Pardo A. and Selman M. (2016). Increased expression of CC16 in patients with idiopathic pulmonary fibrosis. PLoS One 11, e0168552.

Carratt S.A., Kovalchuk N., Ding X. and Van Winkle L.S. (2019). Metabolism and lung toxicity of inhaled naphthalene: Effects of postnatal age and sex. Toxicol. Sci. 170, 536-548.

Chang H., Chang L.W., Cheng Y.H., Tsai W.T., Tsai M.X. and Lin P. (2006). Preferential induction of CYP1A1 and CYP1B1 in CCSP-positive cells. Toxicol. Sci. 89, 205-213.

Chen K., Eddens T., Trevejo-Nunez G., Way E.E., Elsegeiny W., Ricks D.M., Garg A.V., Erb C., Bo M., Wang T., Chen W., Lee J.S., Gaffen S.L. and Kolls J.K. (2016). IL-17 receptor signaling in the lung epithelium is required for mucosal chemokine gradients and pulmonary host defense against K. pneumoniae. Cell Host Microbe 20, 596-605.

Chen L.C., Zhang Z., Myers A.C. and Huang S.K. (2001). Cutting edge: altered pulmonary eosinophilic inflammation in mice deficient for Clara cell secretory 10-kDa protein. J. Immunol. 167, 3025-3028.

Chen Y., Vasquez M.M., Zhu L., Lizarraga R.E., Krutzsch M., Einspahr J., Alberts D.S., Di P.Y.D. Martínez F.D. and Guerra S. (2017). Effects of retinoids on augmentation of club cell secretory protein. Am. J. Respir. Crit. Care Med. 196, 928-931.

Chookliang A., Lanlua P., Niyomchan A. and Sricharoenvej S. (2021). Small bronchiolar histopathological changes related to prolonged diabetes. Int. J. Morphol. *39*, 371-377.

Cohen S.M., Zhongyu Y. and Bus J.S. (2020). Relevance of mouse lung tumors to human risk assessment. J. Toxicol. Environ. Health Part B Crit. Rev. *23*, 214-241.

Dean C.H. and Snelgrove R.J. (2018). New rules for club development: new insights into human small airway epithelial club cell ontogeny and function. Am. J. Respir. Crit. Care Med. 198, 1355-1356.

Donado-Moré A.F., Camargo-Mendoza J.P., Franco-Maz P.G. and Malaver-Caicedo L.F. (2013). La célula de Clara: La biología celular y molecular con implicaciones fisiopatológicas. Una revisión de la literatura. Neumol. Cir. Torax, 72, 306-322.

Dudek S.M., Muñoz N.M., Desai A., Osan C.M., Meliton A.Y. and Leff A.R. (2011). Group V phospholipase A2 mediates barrier disruption of human pulmonary endothelial cells caused by LPS in vitro. Am. Respiratory Cell Mol. Biol. 44, 361-368.

El Agha E. and Thannickal V.J. (2023). The lung mesenchyme in development, regeneration, and fibrosis. J. Clin. Invest. 133, e170498.

Elizur A., Adair-Kirk T.L., Kelley D.G., Griffin G.L., Demello D.E. and Senior R.M. (2007). Clara cells impact the pulmonary innate immune response to LPS. Am. J. Physiol. Lung Cell. Mol. Physiol. 293, L383-L392.

Ge X.N., Chu H.W., Minor M.N., Case S.R., Bosch D.G. and Martin R.J. (2009). Roflumilast increases Clara cell secretory protein in cigarette smoke-exposed mice. COPD 6, 185-191.

Gibbs J.E., Beesley S., Plumb J., Singh D., Farrow S., Ray D.W. and Loudon A.S.I. (2009). Circadian timing in the lung; a specific role for bronchiolar epithelial cells. Endocrinology 150, 268-276.

Gibbs J., Ince L., Matthews L., Mei J., Bell T., Yang N., Saer B., Begley N., Poolman T., Pariollaud M., Farrow S., DeMayo F., Hussell T., Worthen G.S., Ray D. and Loudon A. (2014). An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. Nat. Med. 20, 919-926.

Gowdy K., Krantz Q.T., Daniels M., Linak W.P., Jaspers, I. and Gilmour M.I. (2008). Modulation of pulmonary inflammatory responses and antimicrobial defenses in mice exposed to diesel exhaust. Toxicol. Appl. Pharmacol. *229*, 310-319.

Guerra S., Halonen M., Vasquez M.M., Spangenberg A., Stern D.A., Morgan W.J., Wright A.L., Lavi I., Tarès L., Carsin A.-E., Dobaño C., Barreiro E., Zock J.-P., Martínez-Moratalla J., Urrutia I., Sunyer J., Keidel D., Imboden M., Probst-Hensch N., Hallberg J., Melén E., Wickman M., Bousquet J., Belgrave D.C.M., Simpson A., Custovic A., Antó J.M. and Martinez F.D. (2015). Relation between circulating CC16

concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study. Lancet Respir. Med. *3*, 613-620.

Halatek T., Hermans C., Broeckaert F., Wattiez R., Wiedig M., Toubeau G., Falmagne P. and Bernard A. (1998). Quantification of Clara cell protein in rat and mouse biological fluids using a sensitive immunoassay. Eur. Resp. J. 11, 726-733.

Hay J.G., Claire D., Chu C.S. and Crystal R.G. (1995). Human CC10 gene expression in airway epithelium and subchromosomal locus suggest linkage to airway disease. Am. J. Physiol. Lung Cell. Mol. Physiol. 268, L565-L575.

Hermans C., Petrek M., Kolek V., Weynand B., Pieters T., Lambert M. and Bernard A. (2001). Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis. Eur. Resp. J. 18, 507-514.

Hernández-Santos N., Wiesner D.L., Fites J.S., McDermott A.J., Warner T., Wüthrich, M. and Klein B.S. (2018). Lung epithelial cells coordinate innate lymphocytes and immunity against pulmonary fungal infection. Cell Host Microbe 23, 511-522.

Hiemstra P.S. and Bourdin A. (2014). Club cells, CC10 and self-control at the epithelial surface. *Eur. Resp. J.* 44, 831-832.

Hyde D.M., Plopper C.G., Kass P.H. and Alley J.L. (1983). Estimation of cell numbers and volumes of bronchiolar epithelium during rabbit lung maturation. Am. J. Anat. 167, 359-370.

Hyde D.M., Plopper C.G., Weir A.J. and Pepelko W.E. (1985). Peribronchiolar fibrosis in lungs of cats chronically exposed to diesel exhaust. Lab. Invest. 52, 195-206.

Hung C.H., Chen L.C., Zhang Z., Chowdhury B., Lee W.L., Plunkett B., Chen C.-H., Myers A.C. and Huang S.K. (2004). Regulation of TH2 responses by the pulmonary Clara cell secretory 10-kd protein. J. Allergy Clin. Immunol. *114*, 664-670.

Ince L.M., Pariollaud M. and Gibbs J.E. (2018). Lung physiology and defense. Curr. Opin. Physiol. 5, 9-15.

Itabashi Y., Ravichandran R., Bansal S., Bharat A., Hachem R., Bremner R., Smith M. and Mohanakumar T. (2021). Decline in club cell secretory proteins, exosomes induction and immune responses to lung self-antigens, Kα1 tubulin and collagen V, leading to chronic rejection after human lung transplantation. Transplantation 105, 1337.

Johansson S., Keen C., Ståhl A., Wennergren G. and Benson M. (2005). Low levels of CC16 in nasal fluid of children with birch pollen-induced rhinitis. Allergy, 60, 638-642.

Johnson M.D., Younis U.S., Menghani S.V., Addison K.J., Whalen M., Pilon A.L., Cress A.E., Polverino F., Romanoski C.E., Kraft M., Martinez F.D., Guerra S. and Ledford J.G. (2021). CC16 Binding to $\alpha_4\beta_1$ integrin protects against *Mycoplasma pneumoniae* infection. American J. Respir. Crit. Care Med. 203, 1410-1418.

Karrer H.E. (1956). The ultrastructure of mouse lung: A note on the fine structure of mitochondria and endoplasmic reticulum of the bronchiolar epithelium. J. Biophys. Biochem. Cytol. 2, 115-118.

Kelly F.L., Kennedy V.E., Jain R., Sindhwani N.S., Copeland C.F., Snyder L.D., Eu J.P., Meltzer E.B., Brockway B.L., Pavlisko E., Stripp B.R. and Palmer S.M. (2012). Epithelial clara cell injury occurs in bronchiolitis obliterans syndrome after human lung transplantation. Am. J. Transplant. 12, 3076-3084.

Kim C.F.B., Jackson E.L., Woolfenden A.E., Lawrence S., Babar I., Vogel S., Crowley D., Bronson R.T. and Jacks T. (2005). Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell, 121, 823-835.

Kimura S., Yokoyama S., Pilon A.L. and Kurotani R. (2022). Emerging role of an immunomodulatory protein secretoglobin 3A2 in human diseases. Pharmacol. Ther. 236, 108112.

Kisch B. (1958). Electron microscopy of the lungs in acute pulmonary edema. Exp. Med. Surg. 16, 17-28.

Knabe L., Petit A., Vernisse C., Charriot J., Pugnière M., Henriquet C., Sasorith S., Molinari N., Chanez P., Berthet J.-P., Suehs C., Vachier I., Ahmed E. and Bourdin A. (2019). CCSP counterbalances airway epithelial-driven neutrophilic chemotaxis. Eur. Resp. J. 54, 1802408.

Kokuho N., Ishii T., Kamio K., Hayashi H., Kurahara M., Hattori K., Motegi K., Azuna A., Gemma A. and Kida K. (2015). Diagnostic values for club cell secretory protein (CC16) in serum of patients of combined pulmonary fibrosis and emphysema. COPD 12, 347-354.

Laucho-Contreras M.E., Polverino F., Tesfaigzi Y., Pilon A., Celli B.R. and Owen C.A. (2016). Club cell protein 16 (CC16) augmentation: a potential disease-modifying approach for chronic obstructive pulmonary disease (COPD). Expert Opin. Ther. Targets 20, 869-883.

Lee J.H., Kim J., Gludish D., Roach R.R., Saunders A.H., Barrios J., Woo A.J., Chen H., Conner D.A., Fujiwara Y., Stripp B.R. and Kim C.F. (2013). Surfactant protein-C chromatin-bound green fluorescence protein reporter mice reveal heterogeneity of surfactant protein C-expressing lung cells. Am. J. Respir. Cell Mol. Biol., 48, 288-298.

Lin J., Zhang W., Wang L. and Tian F. (2018). Diagnostic and prognostic values of Club cell protein 16 (CC 16) in critical care patients with acute respiratory distress syndrome. J. Clin. Lab. Anal. 32, e22262.

Liu Y., Yu H.J., Wang N., Zhang Y.N., Huang S.K., Cui Y.H. and Liu Z. (2013). Clara cell 10-kDa protein inhibits T(H)17 responses through modulating dendritic cells in the setting of allergic rhinitis. J. Allergy Clin. Immunol. 131, 387-394.

Liu Z., Liao F., Scozzi D., Furuya Y., Pugh K.N., Hachem R., Chen D.L., Cano M., Green J.M., Krupnick A.S., Kreisel D., Perl A.K.T., Huang H.J., Brody S.L. and Gelman

A.E. (2019). An obligatory role for club cells in preventing obliterative bronchiolitis in lung transplants. JCI insight 4, e124732.

Liu K.Y., Wang L.T., Wang H.C., Wang S.N., Tseng L.W., Chai C.Y., Chiou S.S., Huang S.K. and Hsu S.H. (2021). Aryl hydrocarbon receptor is essential in the control of lung club cell homeostasis. J. Inflamm. Res*.,* 14, 299-311.

López-Valdez N., Guerrero-Palomo G., Rojas-Lemus M., Bizarro-Nevares P., Gonzalez-Villalva A., Ustarroz-Cano M. and Fortoul T.I. (2020). The role of the non-ciliated bronchiolar cell in tolerance to inhaled vanadium of the bronchiolar epithelium. Histol. Histopathol. 35, 497-508.

Macklin C.C. (1949). The two types of epithelium of the finest bronchioles of the albino mouse as revealed by supravital silverization. Can. J. Res. 27, 50-58.

Mariassy A.T., Plopper C.G. and Dungworth D.L. 1(975). Characteristics of bovine lung as observed by scanning electron microscopy. Anatom. Rec. 183, 13-26.

Mariassy A.T. and Plopper C.G. (1983). Tracheobronchial epithelium of the sheep: I. Quantitative light-microscopic study of epithelial cell abundance, and distribution. Anatom. Rec. 205, 263e275.

Martinu T., Todd J.L., Gelman A.E., Guerra S. and Palmer S.M. (2023). Club cell secretory protein in lung disease: emerging concepts and potential Therapeutics. Annu. Rev. Med*. 74*, 427-441.

Miller W.S. (1928). The epithelium of the lower respiratory tract. Cowdrys Special Cytology, 1, 69.

Milne S., Li X., Cordero A.I.H., Yang C.X., Cho M.H., Beaty T.H., Ruczinski I., Hansel N.N., Bossé Y., Brandsma C.A., Sin D.D. and Obeidat M. (2020). Protective effect of club cell secretory protein (CC-16) on COPD risk and progression: a Mendelian randomisation study. Thorax 75, 934-943.

Nord M., Schubert K., Cassel T.N., Andersson O. and Riise G.C. (2002). Decreased serum and bronchoalveolar lavage levels of clara cell secretory protein (CC16) is associated with bronchiolitis obliterans syndrome and airway neutrophilia in lung transplant recipients. Transplantation 73, 1264-1269.

Pack R.J., Al-Ugaily L.H., Morris, G. and Widdicombe J.G. (1980). The distribution and structure of cells in the tracheal epithelium of the mouse. Cell Tissue Res. 208, 65-84.

Pack R.J., Al-Ugaily L.H. and Morris G. (1981). The cells of the tracheobronchial epithelium of the mouse: a quantitative light and electron microscope study. J. Anat. 132, 71-84.

Park H.Y., Churg A., Wright J.L., Li Y., Tam S., Man S.P., Taskin D., Wise R.A., Conett J.E. and Sin D.D. (2013). Club cell protein 16 and disease progression in chronic obstructive pulmonary disease. Am. J. Respir Crit. Care Med. 188, 1413-1419.

Pariollaud M., Gibbs J.E., Hopwood T.W., Brown S., Begley N., Vonslow R., Poolman T., Guo B., Saer B., Jones H., Tellam J.P., Bresciani S., Tomkinson N.C.O., Wojno-Picon J., Cooper A.W.J., Daniels D.A., Trump R.P., Grant D., Zuercher W., Willson T.M., MacDonald A.S., Bolognese B., Podolin P.L., Sánchez Y., Loudon A.S.I. and Ray D.W. (2018). Circadian clock component REV-ERBα controls homeostatic regulation of pulmonary inflammation. J. Clin. Invest. 128, 2281-2296.

Perić A., Mirković C.Š., Đurđević B.V., Perić A.V. and Vojvodić D. (2017). Eosinophil chemokines and Clara cell protein 16 production in nasal mucosa of patients with persistent allergic rhinitis. Eur. J. Med. 49, 178-182.

Plopper C.G., Mariassy A.T. and Hill L.H. (1980a). Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: I. A comparison of rabbit, guinea pig, rat, hamster, and mouse. Exp. Lung Res. 1, 139-154.

Plopper C.G., Mariassy A.T. and Hill L.H. (1980b). Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: II. A comparison of horse, steer, sheep, dog, and cat. Exp. Lung Res. 1, 155-169.

Plopper C.G., Halsebo J.E., Berger W.J., Sonstegard K.S. and Nettesheim P. (1983). Distribution of nonciliated bronchiolar epithelial (Clara) cells in intra- and extrapulmonary airways of the rabbit. Exp. Lung Res. 5, 79-98.

Plopper C.G., Heidsiek J.G., Weir A.J., George J.A. and Hyde D.M. (1989). Tracheobronchial epithelium in the adult rhesus monkey: a quantitative histochemical and ultrastructural study. Am. J. Anat. 184, 31-40.

Plopper C.G., Chu F.P., Haselton C.J., Peake J., Wu J. and Pinkerton K.E. (1994). Dose-dependent tolerance to ozone. I. Tracheobronchial epithelial reorganization in rats after 20 months' exposure. Am. J. Pathol. 144, 404-420.

Plopper C.G. and Hyde D.M. (2015). Epithelial cells of the bronchiole. Comparative Biology of the Normal Lung, 83-92.

Rawlins E.L., Okubo T., Xue Y., Brass D.M., Auten R.L., Hasegawa H., Wang F. and Hogan B.L. (2009). The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell *4*, 525-534.

Reynolds S.D., Giangreco A., Power J.H. and Stripp B.R. (2000). Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am. J. Pathol. 156, 269-278.

Reynolds S.D. and Malkinson A.M. (2010). Clara cell: progenitor for the bronchiolar epithelium. Int. J. Biochem. Cell Biol. 42, 1-4.

Rohmann N., Stürmer P., Geisler C., Schlicht K., Knappe C., Hartmann K., Türk K., Hollstein T., Beckmann A., Seoudy A.K., Becker U., Wietzke-Braun P., Settgast U., Tran F., Rosenstiel P., Beckmann J.H., von Schönfels W., Seifert S., Heyckendorf J., Franke A., Schreiber S., Schulte D.M. and Laudes M. (2023). Effects of lifestyle and associated diseases on serum CC16 suggest complex interactions among metabolism, heart and lungs. J. Adv. Res. S2090, 00168-6.

Rokicki W., Rokicki M., Wojtacha J. and Dżeljijli A. (2016). The role and importance of club cells (Clara cells) in the pathogenesis of some respiratory diseases. Kardiochir. Torakochirurgia Pol. *13*, 26-30.

Roma S.M., Pérez F.A. and D'Ottavio A.E. (2021). El desafiante derrotero de la Histologia médica: Pasado a valorar, presente a construir, futuro a descifrar. Revista Médica de Rosario 87, 98-105.

Sagiv A., Bar-Shai A., Levi N., Hatzav M., Zada L., Ovadya Y., Roitman L., Manella G., Regev O., Majewska J., Vadai E., Eilam R., Feigelson S.W., Tsoory M., Tauc M., Alon R. and Krizhanovsky V. (2018). p53 in bronchial club cells facilitates chronic lung inflammation by promoting senescence. Cell Rep. 22, 3468-3479.

Salwig I., Spitznagel B., Vazquez-Armendariz A.I., Khalooghi K., Guenther S., Herold S., Szibor M. and Braun T. (2019). Bronchioalveolar stem cells are a main source for regeneration of distal lung epithelia *in vivo*. EMBO J. 38, e102099.

Schiller H.B., Montoro D.T., Simon L.M., Rawlins E.L., Meyer K.B., Strunz M., Vieira Braga M., Timens W., Koppelman G., Scott Budinger G.R., Burgess J.K., Waghray A., van den Berge M., Theis F.J., Regev A., Kaminski N., Rajagopal J., Teichmann S., Misharin A.V. and Nawijn M.C. (2019). The human lung cell atlas: a high-resolution reference map of the human lung in health and disease. Am. J. Respir. Cell Mol. Biol. 61, 31-41.

Shijubo N., Itoh Y., Yamaguchi T., Shibuya Y., Morita Y., Hirasawa M., Okutani R., Kawai T. and Abe S. (1997). Serum and BAL Clara cell 10 kDa protein (CC10) levels and CC10-positive bronchiolar cells are decreased in smokers. Eur. Resp. J. 10, 1108-1114.

Shijubo N., Itoh Y., Yamaguchi T., Imada A., Hirasawa M., Yamada T., Kawai T. and Abe S. (1999). Clara cell protein positive epithelial cells are reduced in small airways of asthmatics. Am. J. Respir Crit. Care Med. 160, 930-933.

Singh G. and Katyal S.L. (2000). Clara cell proteins. An. N.Y. Acad. Sci. *923*, 43-58.

Singh G., Katyal S.L., Brown W.E., Kennedy A.L., Singh U. and Wong-Chong M.L. (1990). Clara cell 10 kDa protein (CC10): Comparison of structure and function to uterogloblin. Biochim. Biophys. Acta Enzymol. *1039*, 348-355.

Snyder J.C., Reynolds S.D., Hollingsworth J.W., Li Z., Kaminski N. and Stripp B.R. (2010). Clara cells attenuate the inflammatory response through regulation of macrophage behavior. Am. J. Resp. Cell Mol. Biol. *42*, 161-171.

Stripp B.R., Maxson K., Mera R. and Singh G. (1995). Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. Am. J. Physiol. Lung Cell. Mol. Physiol. 269, L791-L799.

Stripp B.R. and Reynolds S.D. (2008). Maintenance and repair of the bronchiolar epithelium. Proc. Am. Thorac. Soc. *5*, 328-333.

Suarez C.J., Dintzis S.M. and Frevert C.W. (2012). Respiratory system in: Comparative anatomy and histology. Treuting and Dintzis eds. Academic Press. Elsevier Inc. Pp. 121-134.

Tăbăran A.F., O'Sullivan M.G., Seabloom D.E., Vevang K.R., Smith W.E., Wiedmann, T.S. and Peterson L.A. (2019). Inhaled furan selectively damages club cells in lungs of A/J mice. Toxicol. Pathol. 47, 842-850.

Tata P.R., Mou H., Pardo-Saganta A., Zhao R., Prabhu M., Law B.M., Vinarsky V., Cho J.L., Breton S., Sahay A., Medoff B.D. and Rajagopal J. (2013). Dedifferentiation of committed epithelial cells into stem cells *in vivo*. Nature 503, 218-223.

Tiezzi M., Morra S., Seminerio J., Van Muylem A., Godefroid A., Law-Weng-Sam N., van Praet A., Cobière V., Orte-Cano C., Karimi S., del Marmol V., Bondue B., Benjelloun M., Lavis P., Mascart F., van de Borne P. and Cardozo A.K. (2022). SP-D and CC-16 pneumoproteins' kinetics and their predictive role during SARS-CoV-2 Infection. Front. Med. 8, 761299.

Tokita E., Tanabe T., Asano K., Suzaki H. and Rubin B.K. (2014). Club cell 10-kDa protein attenuates airway mucus hypersecretion and inflammation. Eur. Resp. J. 44, 1002-1010.

Tyler N.K. and Plopper C.G. (1985). Morphology of the distal conducting airways in rhesus monkey lungs. Anat. Rec. 211, 295-303.

Umland T.C., Swaminathan S., Singh G., Warty V., Furey W., Pletcher J. and Sax M. (1994). Structure of a human Clara cell phospholipid-binding protein-ligand complex at 1. 9 Å resolution. Nat. Struct. Biol. 1, 538-545.

Verleden G.M., Glanville A.R., Lease E.D., Fisher A.J., Calabrese F., Corris P.A., Ensor C.R., Gottlieb J., Hachem R.R., Lama V., Martinu T., Neil D., Singer L.G., Snell G. and Vos R. (2019). Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment-A consensus report from the Pulmonary Council of the ISHLT. J. Heat Lung Transplant. 38, 493-503.

Voraphani N., Stern D.A., Ledford J.G., Spangenberg A.L., Zhai J., Wright A.L., Morgan W.J., Kraft M., Sherrill D.L., Curtin J.A., Murray C.S., Custovic A., Kull I., Hallberg J., Bergstrom A., Herrera-Luis E., Halonen M., Martinez F.D., Simpson A., Melen E. and Guerra S. (2023). Circulating CC16 and Asthma: A Population-based, Multicohort Study from Early Childhood through Adult Life. Am. J. Respir Crit. Care Med. 208, 758-769.

Wang H.C., Liu K.Y., Wang L.T., Hsu S.H., Wang S.C. and Huang S.K. (2021). Aryl hydrocarbon receptor promotes lipid droplet biogenesis and metabolic shift in respiratory Club cells. Hum. Cell 34, 785-799.

Watson T.M., Reynolds S.D., Mango G.W., Boe I.M., Lund J. and Stripp B.R. (2001). Altered lung gene expression in CCSP-null mice suggests immunoregulatory roles for Clara cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 281, L1523-L1530.

Wiesner D.L., Merkhofer R.M., Ober C., Kujoth G.C., Niu M., Keller N.P., Gern J.E., Brockman-Schneider R.A., Evans M.D., Jackson D.J., Warner T., Jarjour N.N., Esnault

S.J, Feldman M.B., Freeman M., Mou H., Vyas J.M. and Klein B.S. (2020). Club cell TRPV4 serves as a damage sensor driving lung allergic inflammation. Cell Host Microbe 27, 614-628.

Winkelmann A. and Noack T. (2010). The clara cell: a “third reich eponym”? Eur. Respir. J. *36*, 722-727.

Wong A.P., Keating A. and Waddell T.K. (2009). Airway regeneration: the role of the Clara cell secretory protein and the cells that express it. Cytotherapy 11, 676-687.

Yang J., Zuo, W.L., Fukui T., Chao I., Gomi K., Lee B., Staudt M.R., Kaner R.J., Strulovici-Barel Y., Salit J., Crystal R.G. and Shaykhiev R. (2017). Smoking-dependent distal-to-proximal repatterning of the adult human small airway epithelium. Am. J. Respir Crit. Care Med. 196, 340-352.

Yoshikawa S., Miyahara T., Reynolds S.D., Stripp B.R., Anghelescu M., Eyal F.G. and Parker J.C. (2005). Clara cell secretory protein and phospholipase A2 activity modulate acute ventilator-induced lung injury in mice. J. Appl. Physiol. 98, 1264-1271.

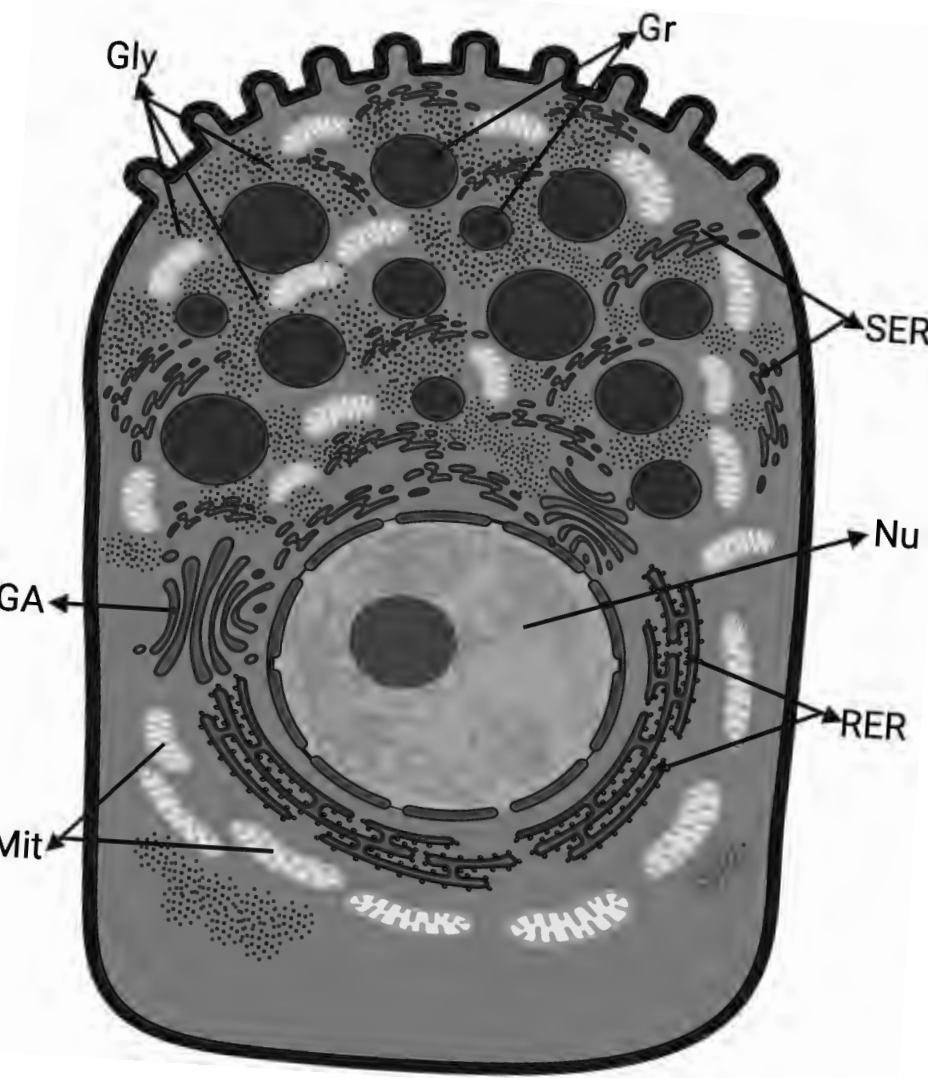
Young S.L., Fram E.K. and Randell S.H. (1986). Quantitative three dimensional reconstruction and carbohydrate cytochemistry of rat nonciliated bronchiolar (Clara) cells. Am. Rev. Respir. Dis. 133, 899-907.

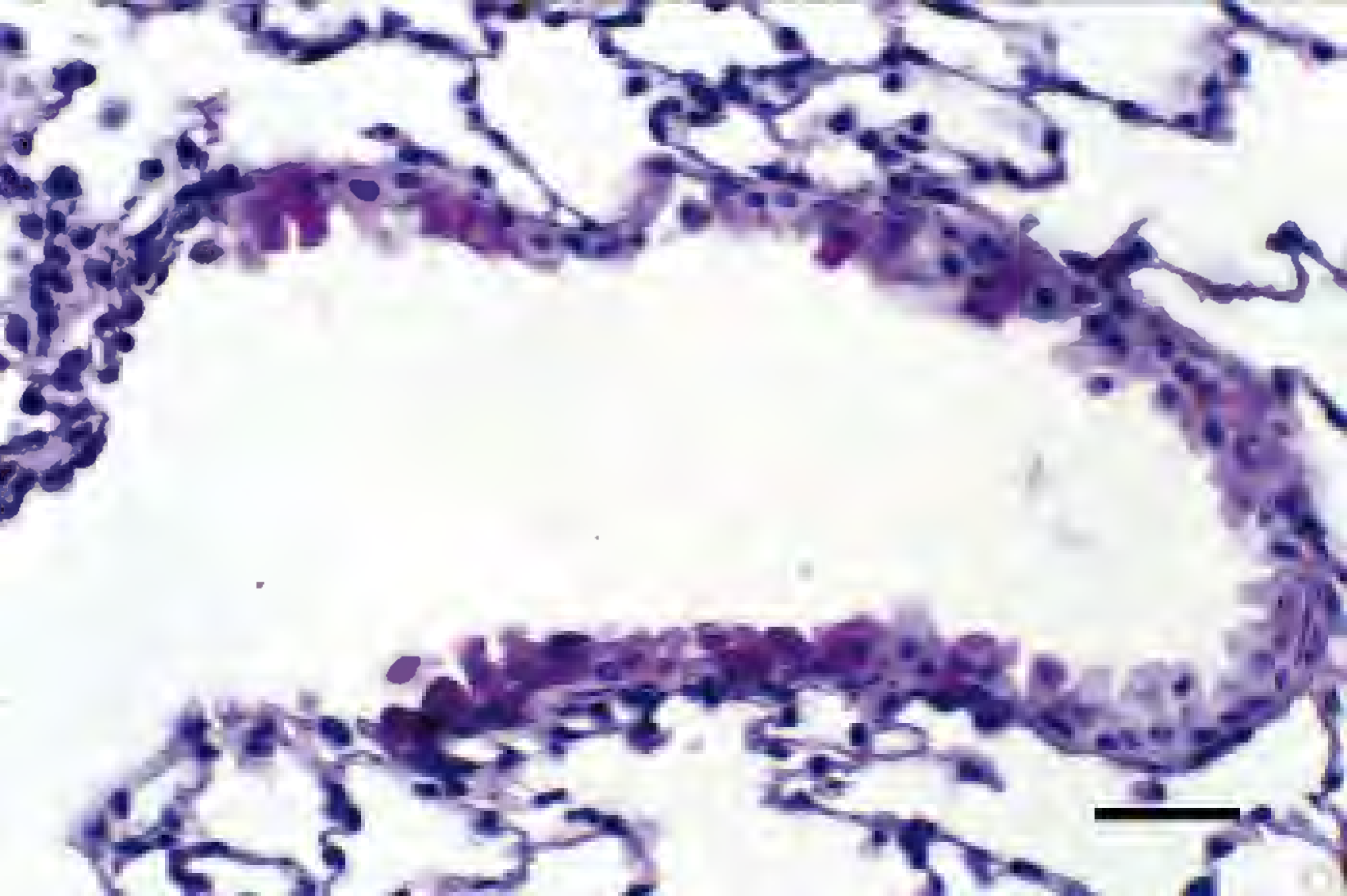
Zhai J., Stern D.A., Sherrill D.L., Spangenberg A.L., Wright A.L., Morgan W.J., Halonen M., Martínez F.D. and Guerra S. (2018). Trajectories and early determinants of circulating CC16 from birth to age 32 years. Am. J. Resp. Crit. Care Med. 198, 267-270.

Zhai J., Insel M., Addison K.J., Stern D.A., Pederson W., Dy, A., Rojas-Quntero J., Owen C.A., Sherrill D.L., Morgan W., Wright A.L., Halonen M., Martínez F.D., Kraft M., Guerra S. and Ledford J.G. (2019). Club cell secretory protein deficiency leads to altered lung function. Am. J. Resp. Crit. Care Med. 199, 302-312.

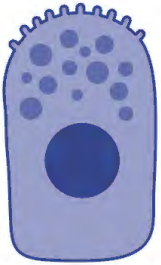
Zhai J., Emond M.J., Spangenberg A., Stern D.A., Vasquez M.M., Blue E.E., Buckingham K.J., Sherrill D.L., Halonen M., Gibson R.L., Rosenfeld M., Sagel S.D., Bamshad M.J., Morgan W.J. and Guerra S. (2022). Club cell secretory protein and lung function in children with cystic fibrosis. J. Cyst. Fibros. 21, 811-820.

Zheng D., Limmon G.V., Yin L., Leung N.H., Yu H., Chow V.T. and Chen J. (2012). Regeneration of alveolar type I and II cells from Scgb1a1-expressing cells following severe pulmonary damage induced by bleomycin and influenza. PloS One 7, e48451.


Gly
Gr
SER
Nu
GA
RER
Mit

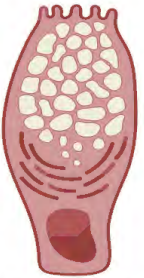


Basal cell

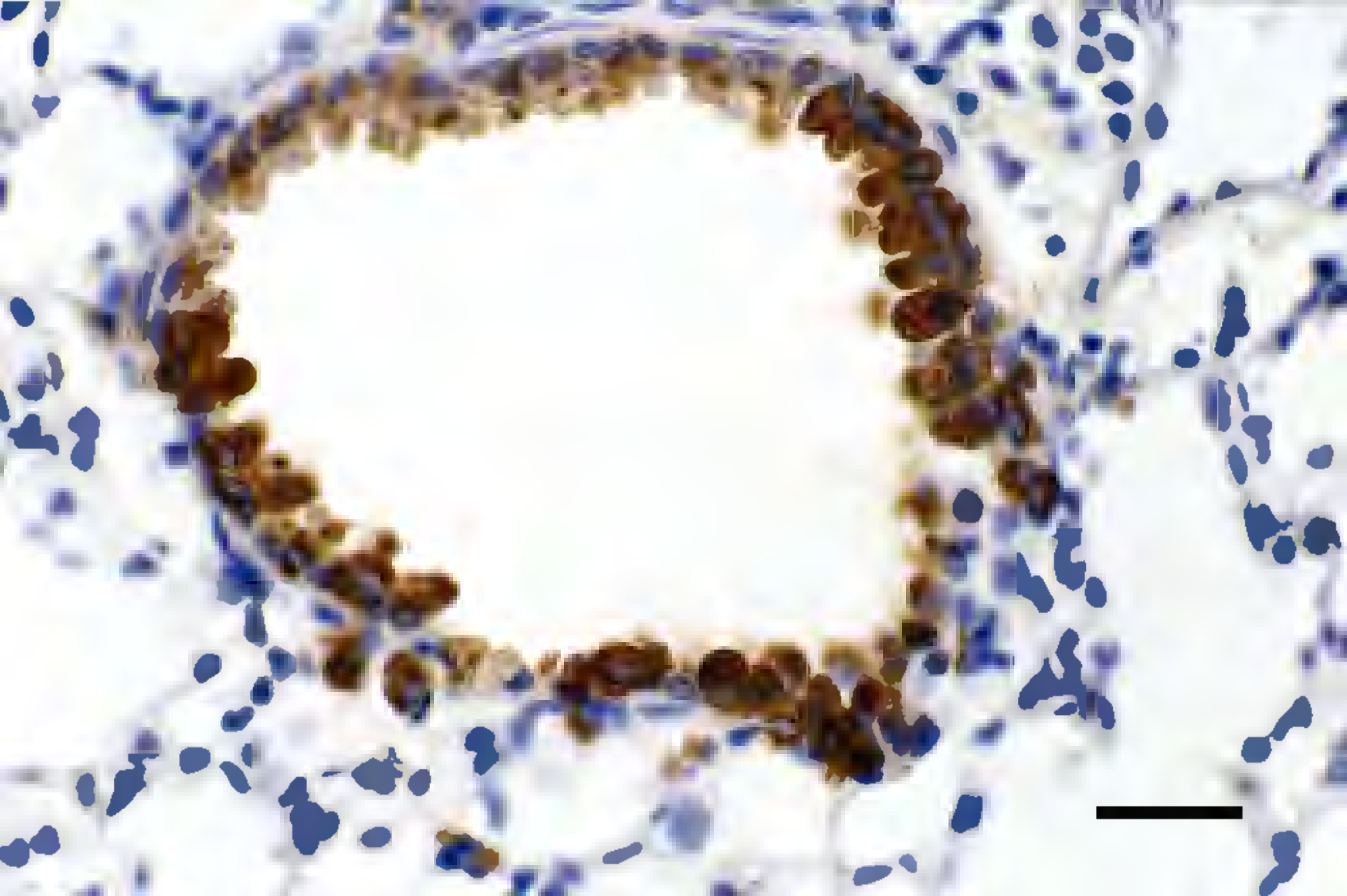


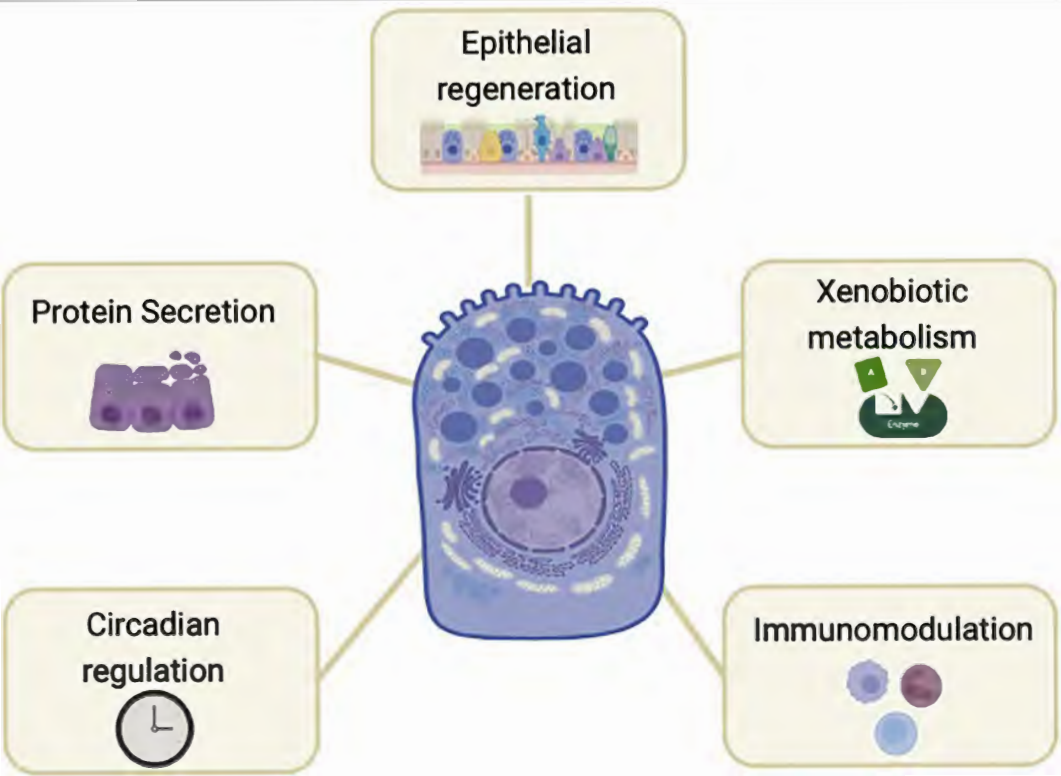
Facultative progenitor
(Club cell)

Ciliated cell



Goblet cell




Epithelial regeneration
Protein Secretion
Xenobiotic metabolism
Circadian regulation
Immunomodulation