EMBJ.ORG FOR YOUNG DOCTORS

EUROMEDITERRANEAN BIOMEDICAL JOURNAL
2020,15 (02) 04–10
(*FORMERLY: CAPSULA EBURNEA*)

Review

THE ROLE OF EMTU IN MUCOSAE REMODELING: FOCUS ON A NEW MODEL TO STUDY CHRONIC INFLAMMATORY LUNG DISEASES

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ARTICLE INFO

Article history:
Accepted 23 November 2019
Revised 13 January 2020
Published 14 February 2020

Keywords:
epithelial-mesenchymal trophic unit, epithelial tissue, mucosae remodelling, chronic respiratory diseases.

ABSTRACT

In recent years, part of the scientific community has focused its attention on the involvement of the epithelial-mesenchymal trophic unit (EMTU) in mucosa remodeling, monitoring its role in chronic inflammatory lung diseases. The term EMTU is used to describe the anatomic and functional relationship between the attenuated fibroblast sheath and epithelial tissue, i.e. the signaling between epithelial cells and the underlying fibroblasts, which are in close indirect physical contact with the former. These interactions are important for many airway functions, such as differentiation during lung growth, repair of damaged tissue and regulation of inflammatory response. Several studies have indicated a key role for the EMTU in the processes that influence mucosae remodelling. These processes can be observed in different pathologies, such as asthma or chronic obstructive pulmonary disease (COPD). This review focuses on the primary role of the EMTU in mucosa remodeling, aiming to give a contribution to an issue that, if deepened may lead to more efficient treatments for the handling of chronic respiratory diseases. The close correlation between the reactivation of the EMTU in adult age and the onset of chronic diseases of the respiratory system is now widely accepted by the scientific community. It is therefore of fundamental importance to enhance our knowledge on its reactivation and the processes that lead to tissue remodeling. Pinpointing the key molecular pathways would provide new therapeutic targets for limiting the remodeling processes typical of subjects suffering from different types of airway pathologies.

1. Introduction

Chronic inflammatory diseases of the lung, such asthma and chronic obstructive pulmonary disease (COPD), are characterized by complex interactions between different cell types, as well as extracellular matrix remodeling. These connections, together with the mechanical stimuli that affect cell-cell and cell-matrix communication, can lead to the development of pathological conditions. Airway remodeling is a significant feature in the pathogenesis of various lung diseases, and it causes persistent changes to the normal architecture of airway walls.

In chronic inflammatory lung diseases, the epithelium has an increased susceptibility to oxidant injury through the activation of the caspase-3/apoptosis pathway [1]. Although the onset mechanisms of asthma and COPD are clearly distinct, these two diseases are both characterized by airway remodeling [2].

The airway remodelling in asthma involves several structural changes, such as a separation of columnar cells from their basal membrane attachments, an increased number of myofibroblasts and abnormally increased collagen deposition within the extracellular matrix (ECM), as well as elevated production of growth factors and pro-inflammatory cytokines [3].

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DOI: 10.3269/1970-5492.2020.15.2
 ISSN: 2279-7165 - Available on-line at www.embj.org

The combined effects of these events, both qualitative and quantitative, will lead, in the long term, to a loss of functional pathways, with a marked reduction in the quality of life of the patient [4].

Past evidence seems to imply that the bronchial epithelium of asthmatic patients is more susceptible to environmental stimuli than a healthy epithelium. One of the causes for this increased susceptibility is the already mentioned atypical production of cytokines and pro-inflammatory interleukins. The main interleukins involved in the pathogenesis of asthma are Th2-type cytokines, such as IL-4, IL-5, and IL-13 [5].

Several mechanisms are involved in the etiopathogenesis of COPD: an increased lymphocyte activity (neutrophils, macrophages, T-cells and B-cells), airway wall remodeling, as well as goblet cell metaplasia and epithelial cell hyperplasia [6]. Even COPD progression has been linked with epithelial-mesenchymal trophic unit (EMTU) activity. Its pathological progression is associated with an evident increase in small airway wall thickness, resulting from a repair and remodeling process involving fibroblast activation within their reactivated epithelial mesenchymal trophic unit [7-9].

Thus, it is clear the pathological together with the clinical relevance of airway remodeling in respiratory diseases and the urgency to assess good models, which may help to study the relationship between microenvironment, EMTU reactivation adults and pathways that are involved in remodeling of the epithelial monolayers.

Furthermore, molecular interactions that lead to airway alterations need to be deepened, through the help of robust and true-to-*in vivo* models.

2. Airways: organogenesis, classic structure and functions

The development of the lungs follows a different timeline compared to the prenatal course of development of other organs, since breathing is not an essential body function inside the uterus. However, lungs must be sufficiently developed to sustain life at birth. Hence, the full course of development of this organ extends all the way from the embryonic period through the fetal one, up until birth. During the intrauterine life of the fetus, lungs are a key source of amniotic fluid. The “inhalation” and “exhalation” of such fluid is essential for normal lung development. Amniotic fluid has also many other important functions, such as protecting the fetus and providing a source of proteins, carbohydrates, lipids, phospholipids, urea and electrolytes, which all contribute to its growth. The lung development process has five distinct phases: the embryonic phase (3^{rd}-8^{th} week), the pseudoglandular phase (8^{th}-16^{th} week), the canalicular phase (16^{th}-24^{th} week), the saccular phase (24^{th}-36^{th} week) and the alveolar phase (36^{th} week- 1/5 years after birth).

After birth, the function of the lungs and, more generally, of the entire respiratory system changes. The properly and fully formed respiratory system consists of a series of organs and anatomical structures whose purposes are ventilation and gas exchange. It has several basic functions: providing a large surface area for gas exchange (to oxygenate the circulating blood), carrying air to and from the lungs through the airways, protecting lung and airway tissue from bacteria, microorganisms and pollutants, as well as from dehydration or other environmental conditions, generating sounds for speech production, enabling odor detection and helping to regulate blood volume and blood pressure (indirectly).

The human lungs, which derive from the main bronchi, represent ideal parenchymal organs.

From an anatomical point of view, the pulmonary lobules can be considered the morpho-functional unit of the lungs.

The connective tissue, divided into septa, defines the structure of these lobules establishing a direct contact with the mesothelium that constitutes the visceral layer of the pleura, thus making the subdivision in septa evident from the outer surface of the organ.

Pulmonary lobules derive from bronchioles, the smallest parts of conducting airways. Bronchioles are characterized by having a diameter smaller than 100 µm with a total absence of cartilage in their walls. The architecture of the pulmonary lobules consists of a terminal bronchiole, which branches into a variable number [3-6, 10] of respiratory bronchioles. From these respiratory bronchioles, the alveolar sacs will originate. The set of respiratory bronchioles and the alveolar sacs associated with it, is called pulmonary acinus. Terminal bronchioles, respiratory bronchioles, alveolar ducts and alveolar sacs develop around the 16th to 28th week of gestation from the expansion and branching of major airways.

In Table 1 are described the different cytotypes that form the epithelium of this tract of the respiratory system. A thin basal membrane is located just underneath the epithelial layer. This membrane is formed by two different laminae: lamina basalis and lamina reticularis; these two structures have a different protein composition, in particular the proteins of the lamina basalis are synthesized by the epithelial cells, and instead the reticularis proteins derive from the connective cells.

Cells	Ultrastructural Features	Functions	Other Putative Roles	Comments
Ciliated cells	Cuboidal, each cell has approximately 250 cilia; each cilium is approximately 6 um long	Transport of mucous stream	Unknown	The most prevalent cytotype; they decrease during chronic inflammation
Clara cells	Cuboidal/columnar non-ciliated, non-mucous secreting cells. Granules are present in apical cytoplasm	Secretory function contributing to the cleaning of smallest airways	Progenitors of other cells (Type II Pneumocytes); role in surfactant production	The second-most prevalent cytotype; they augment during chronic inflammation
Basal cells	Small round cells with minimal cytoplasm, close to the basal membrane	Precursors of other cytotypes	Stemcells	Rare in bronchioles; it is not yet known which cytotypes they originate; also involved in carcinogenesis
Neuroendocrine (Kulchitsky) cells	Small round cells with numerous secretory granules	Part of diffuse neuroendocrine system	Unknown	Rare in bronchioles; may be present single or in small groups; may originate microcytoma
Goblet cells	Cuboidal/columnar mucous secreting cells	Secretory function contributing to the cleaning of smallest airways	Unknown	Rare in bronchioles; they augment during chronic inflammation
Lymphocytes	Small round cells with minimal cytoplasm, scattered among the other cytotypes or the luminal surface of the epithelium	Immune surveillance	Unknown	Rare in bronchioles; they augment during chronic inflammation

Figure 1. Main morphofunctional features of the bronchiolar epithelial cell types and their pathophysiologic roles.

The lamina propria, composed of loose connective tissue, contains smooth muscle cells, mast cells, macrophages, myofibroblasts, fibrocytes, lymphoid cells, endothelial cells of hematic and lymphatic capillaries and nerve fibers. A pool of spindle cells within the lamina propria have been described as fibroblasts, but probably at least some of them belong to a heterogeneous population of undifferentiated cells [11]. Smooth muscle cells are intimately associated with numerous elastic fibers that, together with a small amount of reticular and collagenous fibers, form the feltwork of the lamina propria.

3. Chronic alterations of bronchial mucosa

The airway epithelium represents the interface between the external environment and the airway wall. The normal differentiated bronchial epithelium is a pseudo-stratified structure consisting of a columnar layer with ciliated and secretory cells, supported by basal cells (Figure 1). The epithelium typically releases factors such as prostaglandin (PG) E2 and 15-HETE that suppress mesenchymal cells. Damage to the epithelium has been shown to decrease PGE2 and 15-HETE production, implicating the role of epithelial repair responses in airway remodeling through the activation of the fibroblasts/myofibroblasts located below the epithelial layer. However, the inflammation and the mediator patterns vary in different types of airway diseases, at least in the stable phase of the disease [12]. COPD, like asthma, is a complex inflammatory disease that involves several types of inflammatory cells and multiple inflammatory mediators [13]. In chronic inflammatory lung diseases, airway remodeling results in many structural alterations, including deposition of collagen within the lamina reticularis, thickening of the extracellular matrix in the submucosa, smooth muscle cell hyperplasia and a significant proliferation of microvascular and neuronal cells (Figure 2) [14-15].

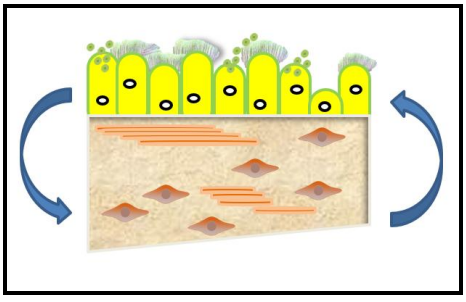

Figure 1. Human healthy respiratory mucosa, with normal exchange of signals between the epithelium and the mesenchymal cells. The ratio between muciparous goblet cells and ciliated elements is correct (around 1:4). The extracellular matrix is of the appropriate thickness and contains the right amount of collagen fibers.

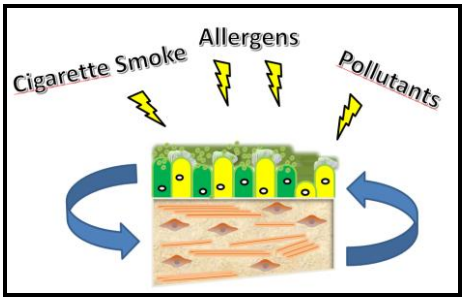

Figure 2. Exposure to environmental insults can lead to airway damage. Increased activation of the epithelium leads to signalling to inflammatory cells activating the underlying mesenchymal cells through the reactivation of the epithelial-mesenchymal trophic unit (EMTU).The modified signaling between mesenchymal and epithelial populations caused an inflammatory state. This condition, over time, leads to tissue changes: increase in the number of muciparous cells, reduction of ciliated cells, thickening of the extracellular matrix, massive deposition of collagen and a continuous release of pro-inflammatory cytokines and chemokines.

Several lines of evidence in animal and human models suggest that the Th2 hypothesis is an incomplete explanation for chronic inflammatory lung diseases [16]. The "Th2 hypothesis" assumes that Immunoglobulin E (IgE) class and eosinophils play specific roles in the pathogenesis of different chronic diseases in the airways; Th2 cytokines modulate IgE synthesis, eosinophil proliferation and their survivability and activity. This “inflamed environment” plays a key role in driving alterations that occur during asthma pathogenesis.

COPD and asthma are both associated with an airflow restriction and a progressive remodeling, affecting the respiratory tract. Henry Hide Salter described asthma for the first time in 1859 as a “disease of reversible airway obstruction” [17]. Most asthmatic patients have a genetic predisposition for the disease. Multiple genes acting either alone or in combination with other genes increase the risk for asthma onset and/or other atopic conditions after exposure to various environmental triggers. Many cells are involved in the pathogenesis of chronic inflammatory lung diseases: lymphocytes, eosinophils and mast cells all seem to play a role in the onset and course of these diseases.

Following a combination of studies conducted on primary cell cultures and biopsies, Holgate and colleagues established that asthma is primarily an epithelial disease, driven by increased environmental susceptibility to injury and by an altered repair response as evidenced by a sustained reactivation of the EMTU that is normally invoked in fetal branching morphogenesis [18-21]. Chronic obstructive pulmonary disease (COPD) is a heterogeneous group of slow-progressing diseases, characterized by airflow limitation and gradual loss of lung function that is not fully reversible. COPD is associated with chronic bronchitis, emphysema and pulmonary hypertension. In clinical practice, COPD is defined by its characteristically low airflow on lung function tests. In contrast to asthma, this limitation is irreversible and progressively worsens over time. The main cause for development of COPD is the inhalation of noxious particles or gases, which trigger an abnormal inflammatory response in the lungs [22]. Most cases of COPD occur as the result of long-term exposure to such irritants. The most common cause for COPD onset is cigarette smoke, however pipe, cigar, and other types of tobacco smoke can also cause COPD, especially if the smoke is inhaled. Breathing in secondhand smoke, air pollution, chemical fumes or environmental dust (often at the workplace) can also contribute to the onset of COPD. Deficiency of alpha-1 antitrypsin (AAT) is another cause of COPD. Macrophages, neutrophils and CD8+ T cells are usually considered the prime effector cells in the pathogenesis of COPD, but recently, dendritic cells have been suggested as a potential new player/orchestrator in the inflammation patterns that characterize this disease. Remodeling correlates with disease severity and the gradual decline of function [23-27].

4. The epithelial mesenchymal trophic unit and airway remodeling: new theories

The anatomical and functional interaction between the attenuated fibroblast sheath and the epithelial layers has been termed the EMTU. The concept of the EMTU was originally introduced by Plopper and Evans [28], and referred to the involvement of the structural airway cells in the regulation of the airway microenvironment during key processes, such as lung development, tissue repair, and inflammatory response.

Stephen Holgate was one of the first researchers to investigate the key role of the EMTU in asthma pathogenesis. Various studies have been conducted on the interactions between the different components of the EMTU [27, 29-31]. *In vitro* studies have shown that damage to epithelial monolayers leads to an increased release of fibro-proliferative and fibrogenic growth factors, including fibroblast growth factor-2, insulin-like growth factor-1, platelet-derived growth factor, endothelin-1, and both latent and active TGF-β2 [31].

In an attempt to identify further factors that could contribute to the increased activity of the EMTU in asthma, A Disintegrin Metalloprotease 33 (ADAM33), a protein codified by a gene on chromosome 20p13, was the first novel asthma susceptibility gene to be described. The positional cloning studies targeting this gene have been successfully replicated in over 33 different population samples worldwide [32-36].

Airway remodeling is a sequence of structural changes that include disruption of epithelial barrier function, sub-epithelial fibrosis, myofibroblast hyperplasia, and smooth muscle cell hypertrophy. Airways remodeling appears to be a complex, multicellular phenomenon, triggered by environmental, age-related, and genetic factors, that causes a progressive decline of respiratory function [37-39].

5. Models to study airway remodeling

The cross talk between the epithelial and fibroblast layer is now well assessed and to enhance our understanding of the changes that occur during the onset and progression of these pathologies, and in particular of the different interactions between epithelial and mesenchymal cells, the scientific community has gradually developed increasingly complex culture models.

The combination of co-cultures and conditioned medium has allowed to deepen the connection between epithelial damage and proliferation of the mesenchymal layer: after an epithelial injury, the culture medium is enriched with pro-inflammatory cytokines and growth factors, which are involved in the proliferation of mesenchymal cells. A direct inhibition of these factors leads to a dramatic reduction of the mesenchymal proliferative potential [40].

Meanwhile, by using conditioned medium it has been further confirmed that the soluble factors released after an epithelial injury are involved in the airway remodeling process. Moreover, it has been noticed that by incubating an intact epithelium with this conditioned medium, the tissue was stuck in a fibrotic phenotype and reparative condition despite not being damaged [40].

2D co-culture models have been widely used for the study of airway remodeling processes; however, this solution has several limitations as the changes in cell morphology, polarity, cell division methods and the altered spatial arrangement of the different cell layers. Thus, with the furthering of the research, the need of more accurate to *in vivo* mockup has been revealed [41].

Current culture models offer varying levels of complexity, but all focus on the importance of obtaining differentiated epithelial cell cultures and being able to observe their interaction with the connective component (directly or indirectly included in the culture model) [16, 42-44].

Modern outgrowth models, such as 3D cultures, allow developing a robust ad true-to in vivo model, in which epithelial layer and fibroblast are in contact and surrounded by their matrix, offering a more representative and truthful reproduction of the complex interactions that take place between different cell populations and their microenvironments *in vivo* [45].

In fact, the main objectives of modern tissue engineering are: fully understanding specific tissue functions that can be applied to regenerative medicine; developing new in vitro models that reproduce specific human tissues; to investigate disease pathogenesis and to test and screen for new therapeutic approaches and drugs before undertaking expensive clinical trials.

6. Pathophysiological remarks

The cell-to-cell communication that drives the physiologic remodeling of the airways during development, when the epithelium and mesenchyme act as a single entity, "trophic unit", is essential to regulate airway growth and branching [46]. Consequently, it is possible that a similar communication takes place during the chronic inflammatory process both in asthma and COPD, where the airway inflammation and remodeling are caused by repetitive environmental injury to a defective airway epithelium due to exposure to viruses, air pollutants, or tobacco smoke (and many other factors whose effects may be amplified by a predisposing genetic condition). Injury leads to interaction between the dysfunctional epithelium and the underlying mesenchyme, resulting in the amplification of inflammatory and remodeling responses in the underlying layers of the airway wall, subsequently impairing the airway repair mechanisms. Thus, the EMTU certainly plays an important role in the development and progression of chronic inflammatory lung diseases, since interaction between the epithelium and fibroblasts have been shown to activate mechanisms that lead to bronchial mucosa remodeling [47-51].The existence of a preserved communication process demonstrates the importance of interactions between epithelial and mesenchymal cells in different anatomical districts.

When this mechanism is altered due to prolonged external factors, the outcome seems to be tissue remodeling with possible development of pathological conditions [52-53].

With the EMTU reactivation, the epithelial cells often undergo epithelial mesenchymal transition (EMT), which plays a crucial role during embryogenic lung formation. Its "reactivation" in adulthood can lead to the onset of COPD, asthma, lung cancer and pulmonary fibrosis. EMT is known to drive key steps in multiple stages of embryonic development: gastrulation, neural crest migration, and heart development. Genetic studies have identified many signaling pathways that can induce EMT, such as transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), nuclear factor-kB (NF-kB), and Wnt [54-56].

Recently, Mahmood and colleagues reported an active role of EMT in invasive non–small cell lung cancer (NSCLC), both in squamous cell and adenocarcinoma cell subtypes; the extent of EMT was strongly correlated with tumor aggressiveness [57].

It can, therefore, be stated that the respiratory mucosa is a major coordinator of the inflammatory response in chronic airway diseases, including asthma and chronic obstructive pulmonary disease (COPD) [58-59]. The inflammatory system has always been considered the main actor in disease development and progression. It has always been thought to play a central role in the immune system, which is certainly involved in the pathological process. However, the results of several studies have implied that the inflammatory system cannot be the exclusive cause for these diseases. In 1991, Howarth and colleagues used immunohistochemistry on samples obtained from bronchial biopsies, describing the inflammatory cells of asthmatic airways.

These infiltrates were characterized by the presence of mast cells, basophils, eosinophils, monocytes, and T lymphocytes with a TH inflammatory profile [60]. Although inflammation undoubtedly plays a central role in asthma, it does not explain many of the characteristic features of this chronic and recurrent disease [21].

Eosinophils have long been thought to play a key role in the pathogenesis of these diseases; however, studies with recombinant human IL-12 [61] or an anti–IL-5 blocking mAb [62-63] have failed to produce positive evidence confirming this hypothesis, despite anti–IL-5 markedly reducing circulating (by approximately 90%), sputum (by approximately 60%-80%), and tissue (by approximately 55%) eosinophil expression. Genetic studies have also demonstrated that atopy and BHR have different patterns of inheritance [4]. These findings imply that local factors play an important role in predisposing individuals to asthma, and could provide an explanation for the epidemiologic evidence that identifies environmental factors, such as pollutant exposure [64-65], diet [66] and respiratory virus infection [67-68] which all increase oxidant stress in the airways, as important disease risk factors.

The importance of airway remodeling, which is the other main histologic characteristic of asthma, in the disease pathogenesis is still a controversial topic since traditionally, inflammation was thought to be the sole cause of asthma and COPD, meaning that airway remodeling has received considerably less attention.

The Childhood Asthma Management Program study in 5- to 11- year-old children showed that the initial beneficial effects of an inhaled corticosteroid on the post-bronchodilator improvement in airway function observed during the first year of treatment was lost over the following 3 years [69].

Although long-standing inflammation has been thought to cause remodeling, airway biopsy studies in young children have shown tissue restructuring up to 4 years before the onset of symptoms [70], indicating that this process begins early on during the development of the pathology, and might occur in parallel with inflammation or even be required for the establishment of persistent inflammation.

7. Conclusions

The paradigm of the “epithelial-mesenchymal trophic unit” in which exaggerated inflammation and remodeling in the airways are a consequence of abnormal injury and repair responses arising from the bronchial epithelium’s susceptibility to components of the inhaled environment, has been proposed to explain the pathogenesis of chronic inflammatory lung diseases. The epithelial-mesenchymal tropic unit is activated during foetal development and is crucial to the process of branching morphogenesis. It has been suggested that similar processes are involved in the structural airway alterations in asthma.

We reviewed the implications of EMTU reactivation in adult hood and the role of EMT during the pathogenesis of chronic lung diseases. From *in vitro* and *in vivo* studies currently in progress, two key notions seem to emerge: (a) functional role of EMT in driving the pathophysiology underlying COPD, bronchiolitis obliterans syndrome (BOS) and idiopathic *pulmonary fibrosis* (IPF), and (b) recognition that EMT may not be a clear-cut binary process with only two endpoints [47].

The possibility of enhancing our knowledge on the mechanisms underlying EMTU activation and mesenchymal epithelial transition may in the future help the scientific community to better understand the onset of many chronic respiratory diseases.

The creation and use of *ex vivo* culture models more and more alike the real architecture of the human respiratory mucosa, coupled with more advanced investigation methods applicable to individuals affected by respiratory diseases, should lead to new insights on the etiopathology of such diseases. If these molecular mechanisms were fully understood, effective treatments to prevent or at least limit the harmful effects of these pathologies would no longer be unattainable.

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