Extracellular Vesicles in Airway Homeostasis and Pathophysiology

Subjects: Pathology

Alberto Fucarino
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Definition

The epithelial–mesenchymal trophic unit (EMTU) is a morphofunctional entity involved in the maintenance of the homeostasis of airways as well as in the pathogenesis of several diseases, including asthma and chronic obstructive pulmonary disease (COPD). The “muco-microbiotic layer” (MML) is the innermost layer of airways made by microbiota elements (bacteria, viruses, archaea and fungi) and the surrounding mucous matrix. The MML homeostasis is also crucial for maintaining the healthy status of organs and its alteration is at the basis of airway disorders. Nanovesicles produced by EMTU and MML elements are probably the most important tool of communication among the different cell types, including inflammatory ones.

1. The Epithelial–Mesenchymal Trophic Unit and the Muco-Microbiotic Layer: Definition, Composition and Functions

Along all the lower airways, except for specific portions, the innermost and proximal layer to the lumen is composed of respiratory mucosa. From a strictly anatomical point of view, in the respiratory mucosa, the outermost layer is made up of a pseudostratified epithelium with mainly goblet and ciliated cells that lie on a basal membrane, below which there is a connective tissue layer with various cells (including fibroblasts, myofibroblasts and immune cells) of mesenchymal origin interspersed in an extracellular matrix (ECM). However, from a morphofunctional point of view, the apical epithelial tissue and the underlying connective tissue within the respiratory mucosa cannot be considered as single, separate entities of their own. Indeed, at the end of the last century, the work of Plopper and Evans focused on the close interconnection between epithelial and mesenchymal cells, providing the basis for the creation of the “epithelial–mesenchymal trophic unit” (EMTU) concept [1]. The role of the interactions between epithelial and mesenchymal elements, although known for some decades at the time of the studies of Evans and Plopper, had been exclusively relegated to airway organogenesis, erroneously assuming that this interaction was limited to intrauterine life [2]. On the contrary, the several cellular and non-cellular components of EMTU in the adult life of individuals are interconnected by a close communicative relationship that influences many other physiological and pathophysiological aspects, such as cell differentiation, tissue homeostasis, organ remodeling, reparative/regenerative processes, response to external/internal stress stimuli and participation in inflammation/autoimmunity. EMTU processes alterations even contribute to the pathogenesis of some chronic diseases of the airways, e.g., chronic obstructive pulmonary disease (COPD) and asthma [3][4][5].

Focusing exclusively on the structural components of EMTU, it must be emphasized that, along with its cellular components, the ECM, synthesized mainly by fibroblasts, also plays a very important role [6]. EMTU homeostasis is frequently influenced by the differences in the lymphocyte population as well as by several soluble factors and nanovesicles dispersed in the ECM that determine the outcome of reparative/regenerative processes and the establishment of pathophysiological states [7][8]. Indeed, it has already been demonstrated that variations in the composition of ECM are the basis of asthma and COPD pathogenesis [9][10][11].

Furthermore, many microbes reside in the mucus constantly produced by goblet cells, constituting the airways’ microbiota. Nowadays, we know that this is another fundamental component for EMTU homeostasis. In the gastrointestinal tract, we already proposed the term “muco-microbiotic layer” (MML) —made by microbiota elements (bacteria, viruses, archaea and fungi) interspersed in a mucous matrix—

to describe the innermost layer of the intestinal wall. The MML homeostasis is crucial for maintaining the healthy status of these organs and whose alteration is at the basis of gastrointestinal disorders [12]. An MML is also present in airways and, as in the gastrointestinal tract, it takes part in the homeostasis as well as in the pathogenesis of these organs. It is fundamental now to better characterize the constitutive elements of this MML in terms not only of microorganisms that populate it but also of nanovesicles (e.g., exosomes, microvesicles or outer membrane vesicles) that participate in the crosstalk among cells.

2. Nanovesicles: Exosomes and Outer Membrane Vesicles

The paracrine communication system regulated by extracellular vesicles (EVs) and exosomes plays a key role in the communication of the EMTU, in the maintenance of tissue homeostasis of the airways as well as in many pathogenetic processes affecting the apparatus [13]. The intimate interconnection that interfaces the epithelial layer to the connective layer, synching them in their biological activities, is favored by the vesicular system that traffics nucleic acids (miRNA, siRNA) [14], growth factors, tissue-specific receptors as well as proteases [15].

As with Evans and Plopper's EMTU studies, extracellular vesicles and exosomes have long been erroneously considered as results of "garbage disposal" whose sole purpose was to eliminate waste substances from cells [16][17]. They are actively secreted by all eukaryotic and prokaryotic cells and are part of an articulated cell-to-cell communication system, both in physiological and pathological conditions [18]. In recent decades, several scientific works demonstrated the roles of EVs in a plethora of physiological processes. EVs are involved in cellular homeostasis and signaling. They can act as carriers of an enormous variety of molecules, and they express numerous signal proteins on their surface [19].

EVs are classified according to their size as (1) microvesicles (100–1000 nm in diameter); (2) apoptotic blebs (1000–5000 nm in diameter); and (3) exosomes (diameter 20–150 nm) [20].

On one side, microvesicles and apoptotic blebs originate from the outward budding of the cell membrane, unlike exosomes, which result from the invagination of endosomal membranes [21]. The understanding of the different originative pathways and the sorting mechanisms spotlighting transport, cargo packing and vesicle exocytosis find practical utility in the isolation studies of EVs for diagnostic and therapeutic purposes.

Therefore, the role of the proteins belonging to the endosomal sorting complex required for transport (ESCRT -0, -I, -II and -III), responsible for the control of the biogenetic and cargo loading processes of the EVs, is crucial [22].

As previously mentioned, the load of the EVs is tissue-specific and related to the function they can perform, i.e., EVs produced by tumor cells have a decisive impact on paracrine signaling mechanisms in support of tumor growth [23][24][25]; however, under physiological conditions, other EVs perform diametrically opposite roles, such as protection from traumatic tissue events or promoting the tissue healing itself [26].

A determining example might be the EVs produced in the lung microenvironment [26]. In homeostasis conditions, a broad range of cell types, such as fibroblasts, epithelial cells and endothelial cells, also actively secrete EVs: those EVs have been largely characterized, showing that epithelial cells are the main characters on the production of EVs, enriched with secretory and membrane-anchored mucins, which contribute to the mucociliary defense and boosting of innate immune defenses [27].

Not only epithelial cells but also macrophages that are present in BAL fluid play a pivotal role in the inflammatory modulation. It has been demonstrated that alveolar macrophages secrete SOCS-1 and -3 within nanoparticles, which are uptaken by lung epithelial cells. Both SOCS-1 and -3 are negative modulators of cytokine 1 and 3 biogenesis (through the STAT pathway inhibition) [28]. In normal conditions, this can modulate the inflammatory response, but at the same time, this negative modulation of IL-1 and -3 biogenesis seems to be lost in cigarette-smoking subjects, presenting a new model for the

control of inflammatory response during inflammation or stress tissue [28].

Thus, with the importance of pulmonary EVs in maintaining homeostasis being confirmed, it is not difficult to think how dysregulations in this sense are closely related to the pathogenesis of various lung diseases [29]. In the following paragraphs, the relationships between microvesicles and chronic diseases of the respiratory system will be analyzed.

3. Conclusions

Asthma and COPD are in themselves very complex and multi-factorial diseases. These pathologies are characterized by airway inflammation, airflow reduction, and airway remodeling. For years, the scientific community has been looking for a solution to this complex puzzle, without success. Probably crucial pieces were missing before they could get the entire picture: the EVs. This carrier is used to transport different molecules and cellular material, not only by our tissues but even by any pathogens eventually present. This review tried to highlight EVs' importance as a biomarker and a potential therapeutic target in two complex chronic airways pathologies: Asthma and COPD. Therefore, an accurate understanding of EVs' roles in these pathologies could lead to a more precise diagnosis and more effective treatments for patients. The contribution made by the microbiome should not be underestimated. The bacterial populations usually present, the opportunistic pathogens, and the possible infections, are all able to condition the microenvironment of the airways through the EVs. The possibility of having additional biomarkers available could be essential to make an early diagnosis or analyze the state of progression of the pathologies. Finally, in the future, EVs pathways analysis may provide new instruments to contrast the chronic respiratory diseases development and progression.

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Keywords

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