Review

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Extracellular vesicles in lung microenvironment and pathogenesis

Yu Fujita[1,2,3], Nobuyoshi Kosaka[1,4], Jun Araya[2], Kazuyoshi Kuwano[2], and Takahiro Ochiya[1]

[1] Division of Molecular and Cellular Medicine, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
[2] Division of Respiratory Diseases, Department of Internal Medicine, Jikei University School of Medicine, 3-19-18, Nishi-shinbashi, Minato-ku, Tokyo 105-8471, Japan
[3] Department of Pathology and Moores UCSD Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA
[4] Department of Zoology, University of Oxford, The Tinbergen Building, South Parks Road, Oxford OX1 3PS, UK

Increasing attention is being paid to the role of extracellular vesicles (EVs) in various lung diseases. EVs are released by a variety of cells, including respiratory cells and immune cells, and they encapsulate various molecules, such as proteins and microRNAs, as modulators of intercellular communication. Cancer cell-derived EVs play crucial roles in promoting tumor progression and modifying their microenvironment. By contrast, noncancerous cell-derived EVs demonstrate protective functions against injury, such as tissue recovery and repair, to maintain physiological homeostasis. Airway cells in contact with harmful substances may alter their EV composition and modify the balanced reciprocal interactions with surrounding mesenchymal cells. We summarize the novel findings of EV function in various lung diseases, primarily chronic obstructive pulmonary disease (COPD) and lung cancer.

Secreted vesicles and intercellular communication in the airway microenvironment

Lung diseases are an increasingly important factor in morbidity and mortality rates worldwide. The high incidence of these diseases generally results from inhalation of air pollution, infectious agents, and various toxic antigens with concomitant immune responses. Airway injury from exposure to cigarette smoke and other air pollutants is a major risk factor in the development of various lung diseases, including lung cancer, pulmonary fibrosis, asthma, and COPD. In healthy subjects, bronchial and alveolar epithelial cells represent the first line of defense against environmental mutagens. The lung epithelium defense system, which is coordinated by effective mucociliary clearance and immune responses, has evolved to maintain proper pulmonary gas exchange function and alleviate toxic substance-induced lung pathology. Repeated exposure of lung epithelial cells to toxic substances (e.g., cigarette smoke) damages the epithelial barrier and eventually induces airway pathology through excessive inflammatory reactions and phenotypic alteration of epithelial cells [1]. Such epithelial damage also activates macrophages, dendritic cells, and innate immune cells, which results in further inflammatory immune responses. Alterations in lung epithelial cells have the potential to change the finely balanced reciprocal interaction between the lung epithelial and mesenchymal cell types that influence cellular differentiation, known as the epithelial–mesenchymal trophic unit (EMTU), which is crucial to lung development [2–5]. In response to injury, aberrant reactivation of the EMTU may contribute to the maintenance of homeostasis or to lung pathology, depending on the nature and duration of the insult, thereby resulting in pathologic airway remodeling in COPD and bronchial asthma. In addition, lung endothelial cells may play a key role in the pathogenesis of mesenchymal and systemic inflammation. Noxious stimuli induce a phenotypic shift in lung endothelial cells through the secretion of inflammatory and chemotactic substances across the capillary–alveolar barrier [6].

Based on these findings, paracrine cell-to-cell communication, primarily orchestrated by lung epithelial cells, is one of the mechanisms underlying physiological homeostasis and disease pathogenesis in the airway microenvironment. However, a complete understanding of cell-to-cell networks in the airway microenvironment is still lacking. By characterizing the representative modalities of communication among lung epithelial cells, endothelial cells, mesenchymal cells, and immune cells, the mechanistic association between the types of injury and airway responses related to subsequently developed airway pathologies may be understood and also allow the identification of novel therapeutic targets [7,8].

In the past decade, extracellular vesicles (EVs) have attracted interest because of their function in intercellular communication [9]. Most cell types, including respiratory cells, release EVs into the extracellular space [10,11]. In 2003, Admyre *et al*. first reported that EVs are present in bronchoalveolar lavage (BAL) fluid [12]. EV contents, which may include DNA, mRNA, microRNA (miRNA), and proteins, are capable of influencing the pleiotropic biological functions of the recipient cells [9]. Therefore, EVs can participate in human physiology and disease pathogenesis as novel paracrine mediators in the

Corresponding author: Ochiya, T. (tochiya@ncc.go.jp).
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airway microenvironment. For example, lung epithelial cell-derived EV cargo may regulate the homeostasis and pathogenesis of the EMTU. In fact, we have recently found that EVs mediate crosstalk between lung epithelial cells and lung fibroblasts, and that smoking-induced epithelium-derived EVs promote myofibroblast differentiation in lung fibroblasts (Y. Fujita *et al.*, unpublished data). Furthermore, EVs can be useful as biomarkers for the diagnosis, prognosis, and therapeutic response of various diseases [13]. In this review we highlight the role of EVs as a representative communication modality among airway cells in modulating lung disease progression and therapeutic applications.

EV classification and biogenesis

EVs were originally described in 1983 in the laboratory of Rose Johnstone [14]. Currently, a growing body of evidence shows that the production of EVs is a universal feature of cellular biological functions. EVs can be detected in cell culture supernatants and in various biological fluids such as blood, urine, sputum, BAL fluid, synovial fluid, pleural effusions, breast milk, and ascites [15]. The main classes of EVs generally include exosomes, microvesicles (also referred to as ectosomes and microparticles), and apoptotic bodies, which are differentiated by their biogenesis and secretion mechanisms [16].

Exosomes are 50–150 nm in diameter and are characterized by their endosomal origin (Figure 1). Exosomes are released by endocytosis following intracellular assembly in multivesicular bodies (MVBs) that contain intraluminal vesicles (ILVs). The endosomal sorting complex required for transport (ESCRT) protein complex is crucial for forming the MVBs and for sorting the endosomal proteins. Then, MVBs can follow either the secretory or lysosomal pathways. Exosomes contain enriched amounts of some specific surface markers, particularly endosomal markers, including tetraspanins (CD9, CD63, CD81), heat shock 70 kDa protein 4 (Hsp70), ALG-2-interacting protein X (Alix), tumor susceptibility gene 101 (Tsg101), and MHC classes I and II [17,18]. Exosomes can transfer their components to target cells through direct membrane fusion, endocytosis, phagocytosis, and ligand/receptor interactions. Figure 2 demonstrates the intracellular biogenesis and the release and uptake mechanisms related to exosomes and other types of EVs.

Microvesicles are shed from the plasma membrane through direct outward budding, and they are larger than exosomes (100–2000 nm). Microvesicles are enriched in phosphatidylserine and contain a membrane component that is similar to that of the parent cell membrane. Exosomes and microvesicles generally contain many cellular components that can affect intercellular communication with recipient cells [9]. These vesicles facilitate cell-to-cell communication through the transfer of functionally relevant biomolecules, including endosome-associated proteins, membrane proteins, lipid raft proteins, and nucleic acids (e.g., DNA, mRNA, and non-coding RNA) [19]. Apoptotic bodies are 1–4 μm in diameter and are released from the plasma membrane as cells undergo apoptosis. Apoptotic bodies may contain DNA fragments, non-coding RNAs, and cell organelles [20].

In recent years, technological methods such as ultracentrifugation, sucrose gradient, ExoQuick precipitation, and immunocapture with anti-EpCam antibodies have been developed to isolate EVs [21]. The gold standard for EV purification is currently ultracentrifugation; however, this is time-consuming and inefficient. Some commercially-available kits that allow easy isolation procedures should be considered with caution because they often fail to distinguish between differently sized EVs and

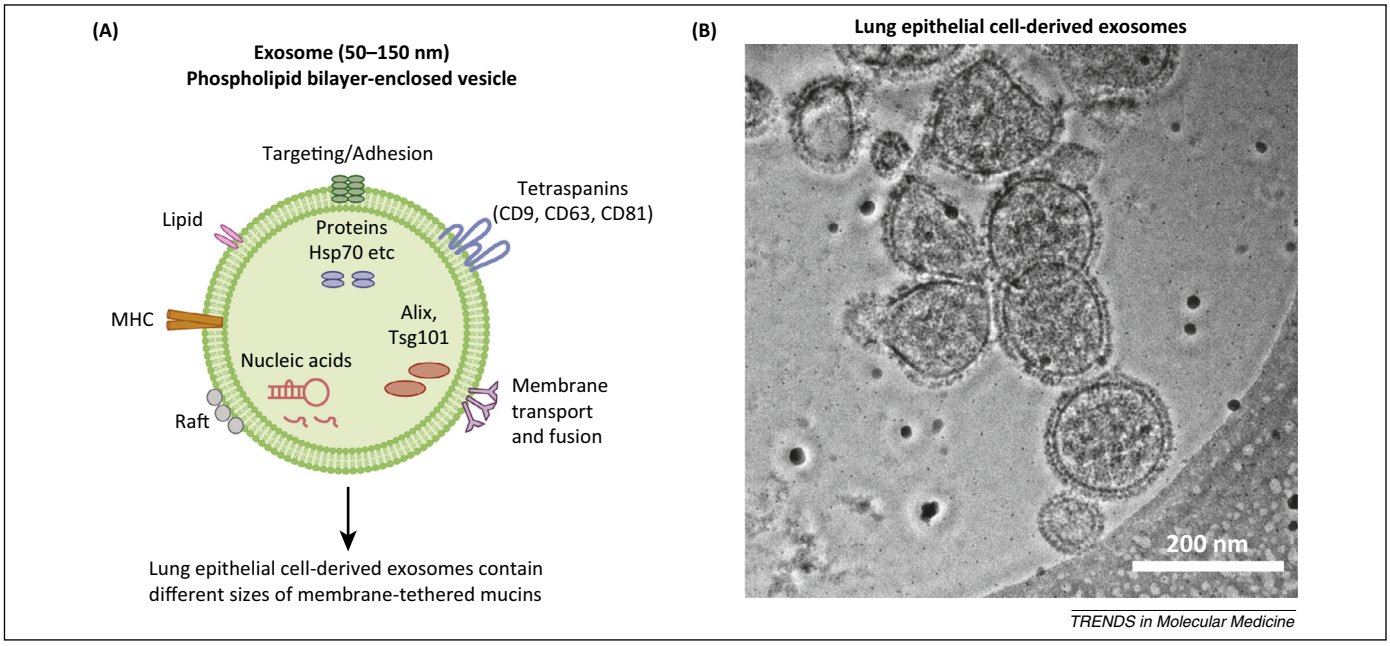

Figure 1. Molecular components of exosomes. **(A)** An exosome is defined as phospholipid-enclosed bilayer. Exosomes contain various proteins on their surface membrane [e.g., tetraspanins, membrane transport and fusion, raft, MHC proteins, and targeting/adhesion molecules]. Cargoes are located inside the luminal space and can include nucleic acids (i.e., DNA, mRNA, and miRNAs) and proteins. Lung epithelial cell-derived exosomes contain different sizes of membrane-tethered mucins that can alter the physical properties of the structure and affect their measured size [26]. **(B)** An electron microscopy image of lung epithelial cell (BEAS-2B)-derived exosomes measuring approximately 50–150 nm in diameter.