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Importance of microbial extracellular vesicle in the pathogenesis of asthma and chronic obstructive pulmonary disease and its diagnostic potential

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ABSTRACT

There are rising evidences of the human microbiome as a potentially influential player that is actively engaged in shaping the pathogenetic processes and other unresolved issues both in asthma and other chronic respiratory diseases, particularly of the airways. The biological components such as microbiome in inhaled air can induce immune dysfunction and inflammation, leading to inflammatory pulmonary disorders such as asthma and chronic obstructive pulmonary disease (COPD). Microbe-derived extracellular vesicles (EVs) with biologically active information or functions can reprogram their respective target cells and EV may have a role for the development of asthma and COPD. To evaluate the role of microbe-derived EV in the pathogenesis of asthma and COPD and its role in diagnosis, the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement method was used for the study. An electronic search was performed using PubMed, PubMed Central, and Embase up to 2020. EVs serve as an intercellular transporter of miRNAs for cell-to-cell communication in the lungs. Bacteria-derived EVs have distinctive characteristics in the lungs of patients with asthma and COPD compared to healthy controls. Furthermore, bacterial EV IgG antibody titers in serum were significantly higher in patients with asthma and COPD than in healthy controls, suggesting that antibacterial EV antibodies titers can be used as a diagnostic tool for lung disease. Taken together, microbial EVs and miRNAs have important roles in the pathogenesis of asthma and COPD and they can provide novel diagnostic biomarkers for asthma and COPD.

Keywords: Microbiome; Extracellular vesicles; miRNA; Asthma; Chronic obstructive pulmonary disease

INTRODUCTION

Microbiota are ecological communities of commensal, symbiotic, and pathogenic microorganisms found in all multicellular organisms from plants to animals. Microbiome is collective genomes of the microorganisms. The microbiome and host emerged during evolution as a synergistic unit from epigenetics and genetic characteristics, sometimes collectively referred to as a holobiont (a host animal and its microbial associates). Changes

Author Contributions

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in the holobiont may impact the complex signaling network thereby influencing the hologenome (the genome of a host animal and its metagenome) leading to health or disease [1-4]. The human microbiome is the aggregate of all microbiota that reside on or within human tissues and biofluids along with the corresponding anatomical sites in which they reside, including the skin, mammary glands, placenta, seminal fluid, uterus, ovarian follicles, lung, saliva, oral mucosa, urine, biliary tract, and gastrointestinal tract. Types of human microbiota include bacteria, archaea, fungi, protists and viruses, most members of which reside in the gastrointestinal tract. Several parameters, including diet, lifestyle, antibiotics and other drugs, hygiene, genetics and immune status of the host, shape the microbiota composition, with various consequences for host physiology [5].

Babies born through the vaginal canal have nonpathogenic, beneficial gut microbiota similar to those found in the mother. However, the gut microbiota of babies delivered by C-section harbors more pathogenic bacteria such as *Escherichia coli* and *Staphylococcus* and it takes longer to develop nonpathogenic, beneficial gut microbiota [6-8]. The Human Microbiome Project (HMP, 2008-2012) was a United States National Institutes of Health initiative to identify and characterize microorganisms found in both healthy and diseased humans. HMP discovered that only 1% of the genes in our bodies are human, the other 99% are contributed by the bacteria in our body, primarily in the gut. Over 10,000 microbial species occupy the human ecosystem. Our body consists of about 40 trillion human cells and about 22,000 human genes. And also, it consists as many as 100 trillion microbial cells and 2 million microbial protein-coding genes. The microbiome consists of microbes that are both helpful and potentially harmful. Most are symbiotic and some, in smaller numbers, are pathogenic. In a healthy body, pathogenic and symbiotic microbiota coexist without problems. But if there is a disturbance dysbiosis occurs, stopping these normal interactions. As a result, the body may become more susceptible to disease [9-11].

There are rising evidences of the human microbiome as a potentially influential player that is actively engaged in shaping the pathogenetic processes and other unresolved issues both in asthma and in the other chronic respiratory diseases, particularly of the airways [12-16].

Extracellular vesicles (EVs) have only recently been recognized as important molecules in the pathogenesis of a number of human diseases particularly, lung diseases. Intercellular communication is an essential hallmark of multicellular organisms and can be mediated through direct cell-cell contact or transfer of secreted molecules. EV is a critical mediator of cell-to-cell communication, which is involved in the physiological and pathological processes of different diseases [17,18]. EVs are nanometer-sized lipid bi-layered vesicles containing cargos from parent cells such as various DNAs, proteins, lipids, mRNAs, and microRNAs (miRNAs). Many diverse names have been used to refer to these vesicles released by healthy cells including ectosomes, microparticles, and shedding microvesicles. Now the term EV are used as a generic term for all secreted vesicles. EVs may be broadly classified into exosomes (endosomal origin, 40–120 nm), microvesicles (plasma membrane origin, 50–1,000 nm), and apoptotic bodies (500–2,000 nm) according to size, structural components, and their origin of generation [19,20]. In the lung, EVs can be released from numerous parent cells both spontaneously and in response to specific stimuli such as inflammation. EVs have emerged as important information shuttles that can coordinate and disseminate homeostatic and disease signals in the lung [21-23].

The biological components such as microbes in indoor dust can induce immune dysfunction and inflammation, leading to inflammatory pulmonary disorders such as asthma and chronic

obstructive pulmonary disease (COPD). Yang et al. [24, 25] reported the importance of indoor dust biological ultrafine particles in the pathogenesis of chronic inflammatory lung diseases. Indoor dust is known to contain EVs derived from microorganisms. Bacteria-derived EVs are spherical, lipid-bilayered vesicles with diameters ranging from 20 to 100 nm, produced by both gram-negative and gram-positive bacteria and are common biological ultrafine particles found in the indoor environment. EVs with biologically active information or functions can reprogram their respective target cells and EV may have a role for the development of asthma and COPD. Moreover, EVs are presently emerging as promising biomarker candidates for a number of lung diseases that currently have little to no reliable means of predicting diagnosis.

The purpose of this review article is to evaluate the role of cell-derived EV, particularly microbial EV relevant to pathogenesis in asthma and COPD and its diagnostic potential as a biomarker for asthma and COPD.

LITERATURE SEARCH

Sources and searches

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement method was used for the study. An electronic search was performed using PubMed, PubMed Central (PMC), and Embase up to 2020. The search key words were EVs, asthma, and COPD. The search collected 26 articles from PubMed, 168 articles with author manuscripts from PMC and 33 articles from Embase.

Selection of articles

In vivo/*ex vivo* studies of biofluids or tissues of patients with asthma and COPD and animal models were selected instead of *in vitro* studies. Titles and abstracts were screened and articles of duplicates, reviews, abstracts, conference proceedings, or articles not relevant to objective were excluded. Only peer reviewed original research articles were included and then 25 articles were included in the final qualitative analysis. Eight of 25 articles investigated the effect of airway exposure on polluted air, microbiome or microbial EVs. Thirteen of them showed the profiles of microbiome, EVs, miRNA in sputum, airway epithelial brushing, bronchoalveolar lavage fluid (BALF) or serum/plasma in asthma and COPD. Two of them showed miRNA spectrum in various body fluids. Two of them provided lung diseases diagnostic model based on IgG antibody titer to microbial EVs.

Among 25 articles 2 were human *in vivo* studies, 16 articles were human *ex vivo* studies, 5 articles were animal experiments, and 2 were combined studies with human *ex vivo* and animal experiments.

MAIN FINDINGS

Biological factors in indoor dust induce chronic inflammatory pulmonary diseases, including asthma and COPD

Asthma and COPD are major lung diseases that cause widespread morbidity and mortality worldwide. The increased prevalence of asthma and COPD patients during the past several decades may be associated with changes in housing styles that have led to increasing amounts of indoor biological contaminants.

Hansel et al. [26] reported that increases in $PM_{2.5}$ concentrations in the main living area were associated with increases in respiratory symptoms, rescue medication use, and risk of severe COPD exacerbations and they concluded that indoor pollutant exposure, including $PM_{2.5}$ was associated with increased respiratory symptoms and risk of COPD exacerbation.

Kim et al. [27] evaluated the hypothesis that airway exposure to different doses of lipopolysaccharide (LPS) induces different form of asthma. We demonstrated that neutrophilic inflammation and IFN-gamma (IFN-γ) expression were higher in induced sputum from severe asthma patients than from mild-to-moderate asthmatics. Animal experiments indicated that allergen sensitization with low-dose LPS (0.1 μg) induced type 2 asthma phenotypes, i.e., airway hyperresponsiveness, eosinophilic inflammation, and allergen-specific IgE up-regulation. In contrast, allergen sensitization with high-dose LPS (10 μg) induced type 1 asthma phenotypes, i.e., airway hyperresponsiveness and noneosinophilic inflammation that were not developed in IFN-γ-deficient mice, but unaffected in the absence of IL-4. Jeon et al. [28] evaluated the effects of double-stranded RNA (dsRNA) on airway sensitization to inhaled allergens in experimental mouse models to see the effects of respiratory viral infections for the development of airway allergen sensitization. We found that lung inflammation enhanced by low-dose dsRNA was impaired in interleukin (IL)-13-deficient mice, whereas lung inflammation by high-dose dsRNA was impaired in IFN-γ-deficient mice. The models also demonstrated that low-dose dsRNA enhanced IL-4 expression during allergen sensitization. In contrast high-dose dsRNA enhanced IFN-γ expression during allergen sensitization. The results showed that airway allergen exposure during respiratory viral infections might induce asthma induced by both Th1 and Th2 immune responses to inhaled allergens. These 2 studies suggest biological factors such as allergens, viruses, and bacterial substances in indoor dust can induce immune dysfunction and chronic inflammation, leading to chronic inflammatory pulmonary diseases, including asthma and COPD.

Microbial diversity is inversely associated with human diseases

The diversity of microbes within a given body habitat can be defined as the number and abundance distribution of distinct types of organisms, which has been linked to several human diseases: low diversity in the gut to obesity and inflammatory bowel disease [29-31]. Ege et al. [32] extracted DNA from mattress dust samples of 489 school-age children from rural and suburban regions in Germany. A fragment of the bacteria-specific 16S ribosomal RNA gene was amplified by polymerase chain reaction, digested to single-strand DNA, and subjected to electrophoresis. They found an inverse association of bacterial diversity with childhood asthma. Thus, diverse microbial environment may account for the protective effect on the development of asthma and atopy.

Microbial EVs induce neutrophilic pulmonary inflammation leading to asthma and COPD

Like mammalian cells, gram-negative and gram-positive bacteria release EVs into the extracellular environment. The contents of gram-negative and gram-positive bacteria-derived EVs have a wide variety of molecules, such as proteins, lipids, DNAs, RNAs, and various virulence factors, which can play important physiological and pathological roles in bacteria-bacteria and bacteria-host interactions [22, 33].

Gram-negative bacteria such as *E. coli* and gram-positive bacteria such as *Staphylococcus aureus* and their microbial EVs were detected in indoor dust. To evaluate whether EVs in indoor air are related to the pathogenesis of pulmonary inflammation and/or asthma Kim et al.

[34] prepared EVs from indoor dust by sequential ultrafiltration and ultracentrifugation. Repeated intranasal application of indoor dust to the experimental mice induced neutrophilic pulmonary inflammation accompanied by lung infiltration of both Th1 and Th17 cells. Kim et al. [35] performed the other animal experiment to evaluate the role of *E. coli*-derived EVs on the development of COPD, such as emphysema. Airway exposure to *E. coli* EVs increased the production of proinflammatory cytokines, such as tumor necrosis factor-α and IL-6. In addition, repeated inhalation of *E. coli* EVs for 4 weeks induced neutrophilic inflammation and emphysema, which are associated with enhanced elastase activity. Emphysema and elastase activity enhanced by *E. coli* EVs were reversed by the absence of IFN-γ or IL-17A genes. Kim et al. [36] performed another animal experiment to evaluate whether inhalation of *Staphylococcus aureus*-derived EV is causally related to the pathogenesis of inflammatory pulmonary diseases. Repeated airway exposure to *S. aureus* EV induced both Th1 and Th17 cell responses and neutrophilic pulmonary inflammation, mainly via a Toll-like receptor 2 (TLR2)-dependent mechanism.

COPD is a chronic inflammatory disease, and bacterial infection may play a role in its pathogenesis. Kim et al. [37] hypothesized that lung EVs might display specific microbiome characteristics in COPD. To test this possibility, they compared the microbiome data from 3 completely age- and sex-matched groups of nonsmokers, healthy smokers and COPD patients. They analyzed and compared the microbiomes of 13 nonsmokers with normal spirometry, 13 smokers with normal spirometry (healthy smokers) and 13 patients with COPD by using 16S ribosomal RNA gene sequencing of surgical lung tissue and lung EVs. They found that bacterially derived EVs have distinctive characteristics in the lungs of nonsmokers, healthy smokers and patients with COPD.

IgG sensitization to microbial EVs in asthma and COPD

Kim et al. [38] measured serum IgG antibodies against dust EVs in 90 healthy control subjects, 294 asthmatics and 242 COPD patients. The results showed that serum IgG antibody level to dust EVs were significantly higher in patients with noneosinophilic asthma, COPD or lung cancer than in healthy control subjects. Adjusted multiple logistic regression revealed that sensitization to dust EVs (high serum antidust EV IgG titer) was an independent risk factor for asthma and COPD which indicate that IgG sensitization to indoor dust EVs appears to be a major risk for the development of asthma and COPD.

Yang et al. [39] performed microbiome analysis of indoor dust EVs isolated from mattresses in apartments and hospitals. We developed diagnostic models based on the bacterial EVs antibodies detected in serum samples via enzyme-linked immunosorbent assay. The levels of antibacterial EV IgG, IgG1, and IgG4 antibodies were found to be significantly higher in patients with asthma and COPD compared to the healthy control group.

Altered miRNA profiles in asthma

Differential expression of several miRNAs in serum and biological fluids (BALF, induced sputum) from asthmatics as compared to healthy controls have been documented.

Maes et al. [40] performed a study to see the association between miRNA expression in sputum supernatants with the inflammatory cell profile and disease severity in asthmatic patients. They investigated miRNA expression in sputum supernatants of 10 healthy subjects, 17 patients with mild-to-moderate asthma, and 9 patients with severe asthma. The results showed that expression of miRNA is increased in sputum of patients with severe asthma and

is linked to neutrophilic airway inflammation, suggesting that these miRNAs contribute to this asthma inflammatory phenotype. Levänen et al. [41] isolated EVs from BALF from healthy control subjects (n = 10) and patients with mild intermittent asthma (n = 10) and EV miRNA was analyzed by using microarrays. They demonstrated that substantial differences in EV miRNA profiles between healthy subjects and patients with unprovoked, mild, stable asthma. These changes might be important in the inflammatory response leading to bronchial hyperresponsiveness and asthma. Gon et al. [42] performed an animal experimental study using house dust mite (HDM) allergen-exposed HDM-sensitized mice and control mice. They isolated airway-secreted EVs from BALF and analyzed the expression of miRNA in EVs or lung tissue using miRNA microarray. The results showed that the amount of EV increased 8.9-fold in BALF from HDM-exposed mice compared with that from sham-control mice. These results indicate that selective sorting of miRNA into EVs and increase release to the airway after HDM exposure would be involved in the pathogenesis of allergic airway inflammation. Zhang et al. [43] explored the expression patterns of miRNA-let 7a, 7b, and 7c in BALF in infants with asthma and airway foreign bodies within the first 8 hours and demonstrated whether the changed expression of miRNA-let 7a, 7b, and 7c in BALF were related with the asthma of infants. They found that the increased expressions of miRNA-let 7a, 7b, and 7c in BALF from infants were related to the asthma, not airway foreign bodies. This study indicates that expression levels of miRNA-let 7a, 7b, and 7c might be potential biomarkers for distinguishing asthma and airway foreign bodies in infants. Solberg et al. [44] evaluated whether airway epithelial miRNA expression is altered in asthma. They used miRNA microarrays to analyze bronchial epithelial brushings from 16 steroid-naive subjects with asthma before and after inhaled corticosteroids, 19 steroid-using subjects with asthma, and 12 healthy control subjects. The results showed that dramatic alterations of airway epithelial cell miRNA levels are a common feature of asthma.

To determine whether miRNAs are differentially expressed in asthma, Panganiban et al. [45] isolated serum from 10 asthmatics and 10 control subjects for miRNA profiling. They showed differential serum expression patterns of miRNA in asthmatic patients compared to nonasthmatic controls, demonstrating the potential of miRNA profiling in the diagnosis and management of asthma.

Altered miRNA profiles in COPD

COPD causes significant morbidity and mortality worldwide and is expected to become the third leading cause of death by 2020. miRNAs are small RNA molecules (approximately 21–25 nucleotides long) that negatively regulate gene expression posttranscriptionally, by means of mRNA degradation, inhibition of protein translation, or by a combination of both mechanisms. miRNAs are predicted to regulate approximately 60% of all human protein-coding genes. De Smet et al. [46] discussed the miRNA expression patterns in lungs of patients with COPD and in mouse models and they highlighted various miRNAs are involved in the development and progression of COPD. Van Pottelberge et al. [47] performed a study to identify miRNA expression in induced sputum and examined whether the expression of miRNAs differed between patients with COPD and subjects without airflow limitation. Eight miRNAs were expressed at a significantly lower level in current-smoking patients with COPD compared with never-smokers without airflow limitation. Schembri et al. [48] performed a study to determine whether miRNAs play a role in regulating the airway gene expression response to smoking. They examined whole-genome miRNA and mRNA expression in bronchial airway epithelium from current and never-smokers and found 28 miRNAs to be differentially expressed with the majority being down-regulated in smokers.

EV and miRNA in exacerbation of asthma and COPD

Bacterial and viral infections are common causes of exacerbations of respiratory diseases such as asthma and COPD. miRNAs are a class of small single-stranded RNA that is involved in gene expression regulation at posttranscriptional level of both innate and adaptive immune response to viral infections which suggests their potential contribution to the pathogenesis of asthma exacerbation [49].

Wardzyńska et al. [50] studied 21 asthmatics during asthma exacerbation. They reported that the expression of circulating miRNAs during asthma exacerbation was associated with the objective parameters of disease severity. And they documented that asthma exacerbation is associated with epigenetic dysregulation expressed by changes in circulating miRNA. Kho et al. [51] identified, in the sera of children with asthma, 12 miRNAs that were significantly associated with exacerbations in the subsequent year, with each doubling of expression of these miRNAs associated with a 25%–67% increase in risk of exacerbations. This study suggests that the assessment of the expression of these miRNAs in serum has the potential to identify reliable biomarkers for asthma exacerbation. Eltom et al. [52] hypothesized that respiratory infections cause the release of EVs in the airway, triggers neutrophilia and subsequent disease exacerbations. To test this hypothesis, they utilized human cell-based assays, *ex vivo* murine BALF, *in vivo* preclinical models and human samples. Data showed that infective challenge causes exacerbated inflammation and infection can trigger the release of EVs. This study suggests a possible mechanism for how infections could exacerbate respiratory diseases.

EVs and miRNA as potential biomarkers for asthma and COPD

EVs are found in circulation and contain cell-derived biomolecules. Kadota et al. [22] pointed out that EV has been highlighted as a new disease biomarker for 3 reasons. (1) EVs reflect the physiological state and microenvironment of their cells of origin, and most cells secrete EVs containing specific proteins and nucleic acids; (2) EVs are found in the blood, urine and other body fluids; (3) EVs are very stable in the extracellular environment after their release from cells because of the phospholipid bilayer. Circulating miRNAs are also stable and protected from ribonucleases. Kadota et al. [22] analyzed the miRNA profile in the plasma or sputum for risk prediction and diagnosis of COPD. Nagano et al. [23] revealed EVs in BALF from asthma patients could be biomarkers of asthma.

Weber et al. [53] examined the presence of miRNAs in 12 human body fluids and urine samples from women in different stages of pregnancy or patients with different urothelial cancers. The results showed that extracellular miRNAs are both present and stable in a diverse array of extracellular body fluids including blood serum/plasma, BALF, saliva, peritoneal fluid, pleural fluid, cerebrospinal fluid, and urine. They suggested that extracellular miRNAs can be used as informative biomarkers to assess and monitor the body's physiopathological status. Yeri et al. [54] isolated total extracellular RNA (exRNA) and sequenced from 183 plasma samples, 204 urine samples, and 46 saliva samples from 55 male college athletes ages 18–25 years. They found that microbial exRNAs also circulate in the human body and high levels of bacterial RNA fragments exist in human biofluids.

Sundar et al. [55] used different EV isolation and purification methods to characterize the plasma-derived EV miRNAs from nonsmokers, smokers, and patients with COPD. They found that plasma-derived EVs from nonsmokers, smokers, and patients with COPD vary in their size, concentration, distribution, and phenotypic characteristics. And they concluded that plasma-

derived EV miRNAs are novel circulating pulmonary disease biomarkers. Thus, molecular profiling of EV miRNAs has great translational potential for the development of biomarkers that may be used in the diagnosis, prognosis, and therapeutics of COPD. Xie et al. [56] analyzed serum miRNA profiles in 41 healthy controls, 40 asymptomatic heavy smokers, and 49 COPD patients. The results showed that the levels of serum miR-21 and miR-181a in asymptomatic heavy smokers and COPD patients were significantly higher than in healthy control patients. They suggested that the levels of serum miR-21 and miR-181a and their ratio have potential biomarker utility for predicting the development of COPD in heavy asymptomatic smokers. Extracellular miRNAs may be useful as biomarkers for allergic disease, with the ability to classify disease subtype or activity, and that biologically relevant extracellular miRNAs may contribute to the pathogenesis of allergic inflammation and asthma [57]. Yang et al. [39] provided a lung disease diagnostic model based on antibacterial EV antibody titers.

SUMMARY AND DISCUSSION

The number of microbial cells in human body surpasses the number of actual human cells. Our body consists of about 40 trillion human cells and also it consists as many as 100 trillion microbial cells. Our bodies interact with trillions of microorganisms in our body that are capable of complex biological reactions. The interactions between microbes and human cells appears to have coevolved and this coevolution has likely shaped evolving phenotypes in all life forms [58, 59].

Intercellular communication between microbe and human is essential for the homeostasis of biological systems and it is one of the key mechanisms of lung disease biology. There are rising evidences that microbial EVs and miRNAs are essential for microbe-host communication as they can modulate the expression of host genes. EV may serve as an intercellular transporter of miRNAs for cell-to-cell communication in the lungs. miRNAs are endogenous, single-strand, noncoding RNAs, 20 to 23 nucleotides in length, that regulate translation through their interactions with mRNA transcripts [60]. Thus, microbial EVs and miRNAs may represent key signaling molecules that facilitate relationship between host and microbiome. Interaction between human hosts and the microbiome occurs through a number of mechanisms, including transcriptomic regulation by miRNA [61].

EVs and miRNA from both gram-positive [62] and gram-negative bacteria [63] have been observed to invade host cells, suggesting their possible function as communication molecules that influence host cell functions. Which indicate that host gene regulation by microbial EV and miRNA is a novel pathogenic mechanism, and that the transfer of microbial EV and miRNAs to host cells represents an additional example of microbe-host interaction. Kim et al. [34-36] reported that airway exposure to gram-negative and gram-positive bacteria-derived EVs in indoor dust can induce neutrophilic pulmonary inflammation, asthma and COPD. The ability of EVs to circulate through the bloodstream and reach any place in the human body implies that EVs and their components such as miRNA are relevant to human diseases [64]. Bacteria-derived EVs can affect host immunity with pathogenic bacteria-derived EVs having proinflammatory effects of host immune cells while probiotic derived EVs mostly shape the immune response towards tolerance [65].

In viral infections, EVs produced by infected cells could be a central player in disease pathogenesis. EVs have emerged as specific carriers of cellular and viral components, including miRNAs,

proteins, and viral genomes, and they can be produced during both active viral replication and during viral latency [66]. Internalized viruses have been demonstrated to express miRNAs. Many viruses encode miRNAs that are expressed in host cells, and these miRNAs facilitate viral replication and survival, and suppress or regulate host immunity [67, 68]. The miRNAs play a well-documented regulatory role in controlling functions of cells associated with airway inflammation and are strongly modulated by viral infections, which suggests their potential contribution to the pathogenesis of asthma exacerbation [49]. Wardzyńska et al. [50] reported that the expression of circulating miRNAs during asthma exacerbation was associated with the objective parameters of disease severity. Eltom et al. [52] revealed that respiratory infections causes the release of EVs in the airway, triggers neutrophilia and subsequent disease exacerbations of asthma and COPD.

COPD is one of the major causes of mortality and morbidity worldwide. Among several risk factors for COPD, cigarette smoking is the main factor. Smokers have a higher prevalence of lung function abnormalities and a greater COPD-related mortality rate than nonsmokers [69, 70]. However, smoking does not explain all of the aspects of this condition, because COPD can develop in nonsmokers, and more than half of smokers do not have COPD [71]. Bacterial infection plays a role in pathogenesis of COPD. Bacteria secrete EVs, which may induce more immune dysfunction and inflammation than the bacteria themselves. Kim et al. [37] found that bacterially derived EVs have distinctive characteristics in the lungs of nonsmokers, healthy smokers and patients with COPD. This information should contribute to knowledge of the involvement of lung microbiome in the development of COPD. Yang et al. [39] demonstrated that the levels of antibacterial EV IgG antibodies were higher in patients with asthma and COPD compared to the healthy controls and we provided a lung disease diagnostic model based on antibacterial EV antibody titers.

Taken together, microbe-derived EV and miRNA may play vital roles in the pathogenesis of asthma and COPD and can provide novel diagnostic biomarkers for asthma and COPD, especially useful in possible early detection of these lung diseases.

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