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Review

Exosomes: A new approach to asthma pathology

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ABSTRACT

Asthma is a chronic inflammatory disease of the airways with a complex pathophysiology, making the development of diagnostic and therapeutic tools a challenge. Exosomes are extracellular membranous nanovesicles implicated in intercellular communication. Exosome composition and cargo are highly heterogeneous depending on their cellular origin and physiological state. They contain proteins (tetraspanins, heat-shock proteins), nucleic acids (RNA, microRNA), and lipids (ceramides, cholesterol, sphingolipids). Current scientific advances show that exosomes play a pivotal role in the pathology of asthma as well as other inflammatory diseases, and all types of inflammatory cells (neutrophils, dendritic cells, lymphocytes, eosinophils) release exosomes. Also, structural lung cells such as airway epithelial cells and airway smooth muscle cells produce and secrete these nanovesicles. Exosomes influence and modify the functionality of these inflammatory and structural cells, triggering the characteristic processes of asthma disease. Additionally, exosomes are used as biomarkers in several disorders because they are easier to collect from different biofluids, making them a non-invasive method for screening human pathologies. Also, due to their special molecular characteristics, they can be loaded with different molecules and employed as a drug-delivery vehicle. This review focuses on recent advances related to the role of exosomes in asthma disease.

1. Introduction

Asthma is one of the most prevalent chronic diseases in the world. According to estimates, it affects more than 315 million people worldwide, with nearly 10% of all asthma patients presenting severe or uncontrolled forms of the disease [1,2]. Asthma is characterized by recurrent episodes of wheezing, dyspnoea, cough, and chest tightness. These symptoms are triggered by inflammatory and structural changes causing airflow limitation and bronchial hyperresponsiveness to a wide range of environmental stimuli [3,4]. Recently, asthma has been defined as a complex disease or syndrome and can be stratified into several phenotypes and endotypes, each having specific clinical and pathological characteristics and responding differently to pharmacological treatment [5,6].

This complexity is due to the participation of different cell types in the pathogenesis of asthma: structural lung cells, dendritic cells, T and B lymphocytes, monocytes, eosinophils, *etc.* [7]. In this context, underlying cell-to-cell communication is key, and is mainly performed by soluble mediators such as cytokines, chemokines, and other soluble factors. Nowadays, however, extracellular vesicles (EV) have become recognized as new players in this process. A variety of these EV have now been identified, exosomes being one of them.

Exosomes are small vesicles (30–100 nm in diameter) that enable cell-to-cell communication by shuttling different molecules such as nucleic acids (DNA, mRNA, and microRNAs [miRNA]), lipids, proteins, and specific cell-surface markers that reflect the exosome-cell origin [8]. They deliver this molecular cargo to the target cells, which may alter cell function and behaviour over short or long distances.

Abbreviations: AEC, airway epithelial cells; APC, antigen-presenting cells; BALF, bronchoalveolar lavage fluid; BSMC, bronchial smooth muscle cells; DC, dendritic cells; ECP, eosinophil cationic protein; EPO, eosinophil peroxidase; EV, extracellular vesicles; FEV_1, forced expiratory volume in 1 s; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; IL, interleukin; ILV, intraluminal vesicles; LBPA, lysobisphosphatidic acid; LFA, lymphocyte function-associated antigen; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LT, leukotrienes; MBP, major basic protein; MDM, monocyte-derived macrophages; MHCII, major histocompatibility complex class II; miRNA, microRNAs; MMP, metalloproteinase; MSC, mesenchymal stem cells; MVB, multivesicular bodies; NO, nitric oxide; NOS2, nitric oxide synthase 2; PBMC, peripheral blood mononuclear cells; PGE_2, prostaglandin E_2; ROS, reactive oxygen species; SAEC, small airway epithelial cells; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cells; Tsg101, Tumor Susceptibility Gene 101; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor

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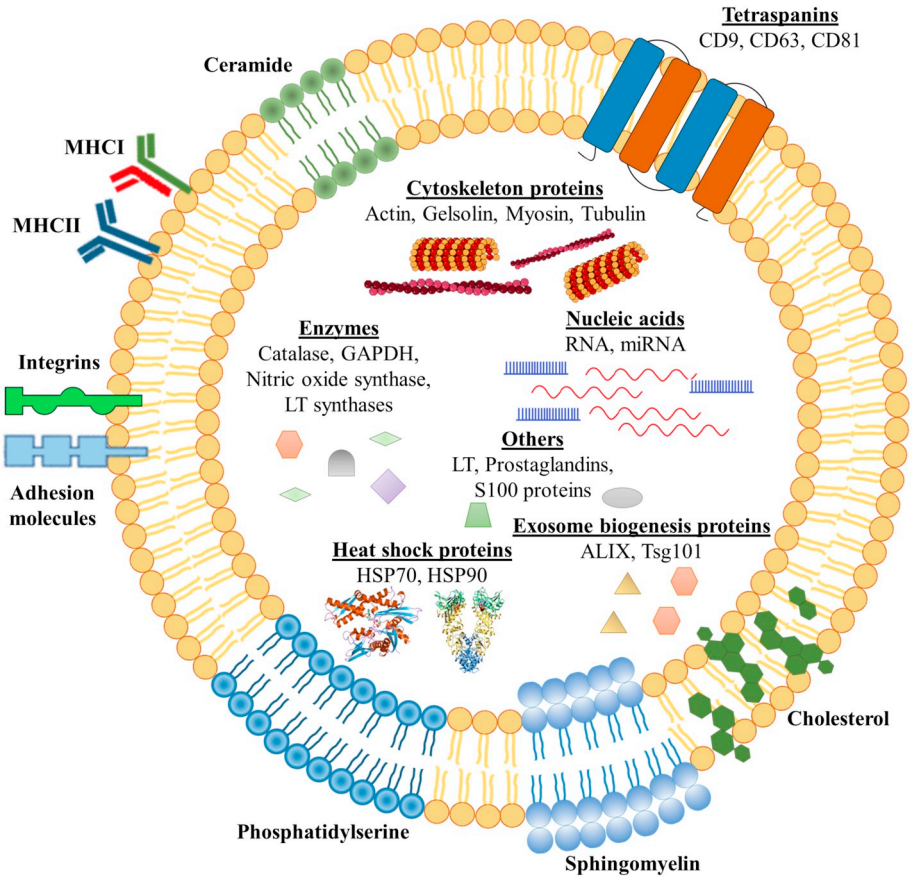

Fig. 1. General structure and molecular composition of an exosome. These nanovesicles contain a wide spectrum of molecules, such as lipids, proteins, and nucleic acids (RNA and miRNA). Exosomes are implicated in intercellular communication and their cargo can be transferred from origin cells to target cells. Exosomes are composed of a lipid bilayer, which is enriched in sphingolipids (sphingomyelin), phosphatidylserine, cholesterol, and ceramides. They also contain several types of proteins, such as endosomal origin proteins (ALIX, Tsg101), tetraspanins (CD9, CD63, CD81), heat-shock proteins (HSP70, HSP90), enzymes (GAPDH, nitric oxide synthase, catalase), cytoskeleton protein (actin, gelsolin, myosin, tubulin), adhesion molecules, integrins, MHCI and MHCII, and others (leukotrienes, prostaglandins, S100 proteins). GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSP, heat shock protein; LT, leukotrienes; MHCI, major histocompatibility complex class I; MHCII, major histocompatibility complex class II; miRNA, microRNAs; Tsg101, Tumor Susceptibility Gene 101.

Exosomes have been extensively studied to elucidate their association with the pathogenesis of a range of inflammatory diseases [9–11] as well as in respiratory diseases [12–15]. These studies have produced fundamental insights into the mechanisms of cellular crosstalk in asthma [16–18]. In this review we summarize recent advances concerning the role of exosomes in the pathogenesis of asthma and discuss their potential as biomarkers and as therapeutic tools.

2. Exosome composition and function in asthma

Exosomes are membranous nanovesicles made up of a lipid bilayer that protects the different bioactive molecules contained within exosomes from the hostile microenvironment [19]. The intraluminal cargo is extraordinarily complex and varies based on cell type and pathophysiological state. In recent years, many studies have explored the nature of exosome molecular composition [20]. Fig. 1 shows a general scheme of exosomal molecular composition.

In this part of the review, we focus on the different components of exosomes (proteins, lipids, and miRNA) and their function in asthma disease.

2.1. Exosomal proteins

Exosomes have characteristic proteins in their proteomic composition. Some are common to all exosome types independently of their origin, such as tetraspanins (CD63, CD9, CD81), heat-shock proteins (HSP70, HSP90), Tumor Susceptibility Gene 101 (Tsg101), ALIX, actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), among others. These proteins are often used as exosome markers [21]. However, some proteins are specific and depend on cell origin. Using mass spectrometry and Western blotting, our group recently demonstrated that eosinophil-derived exosomes contain eosinophil-specific proteins such as eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and major basic protein (MBP) [17].

Though many studies have been performed to describe exosomal structural proteins and these nanovesicles are known to play an important inflammatory role in asthma pathogenesis, investigations showing a concrete relation between asthma and exosome proteins are scarcer. In one notable exception, Rollet-Cohen et al. recently performed comparative proteomic research in exosomes [22]. To do this, they used bronchoalveolar lavage fluid (BALF)-derived exosomes from paediatric patients with asthma, cystic fibrosis, and primary ciliary dyskinesia, finding 14 proteins that were deregulated among these three disease-related groups. These proteins were associated with inflammatory processes such as LCN2 and S100A12. Similarly, in 2016, Lässer et al. compared the exosomal proteome of nasal lavage fluid between healthy individuals, asthmatics, and patients with asthma and chronic rhinosinusitis [23]. For first time, the authors reported the presence of nitric oxide synthase 2 (NOS2) enzyme in exosomes, which increases nitric oxide (NO) levels in inflammatory pathologies. Moreover, they found that filaggrin and hornerin are decreased in exosomes from asthmatics compared to healthy subject-derived exosomes, possibly due to the loss of integrity and dysfunction of the epithelial barrier in asthma pathogenesis.

Another important feature of exosomes is that their surface proteins are critical for (i) establishing connections between the plasma membrane or extracellular matrix and cellular uptake, (ii) exosome mobility, and (iii) immune recognition [24]. Exosome-surface proteins are important in interactions with cells and trigger signal transduction or produce uptake by the cells. Generally, tetraspanins (CD9, CD63, and CD81), integrins, cell-adhesion molecules, proteoglycans, and lectins have been related to these processes [25]. Within asthma pathology, our group and other authors showed that exosomes can be taken up by cells, leading to the hypothesis that the most probable mechanism is

through endocytosis [17,18,26]. However, no research has concretely described the specific mechanism and molecules that participate in this interaction and uptake, thus creating a need for further in-depth studies to explain this phenomenon.

2.2. Exosomal lipids and lipid mediators

Lipids constitute a fundamental part of exosome composition and are related to exosome biogenesis. To date, however, exosomal lipids remain under-researched.

Lipids participate in exosome formation and are important in early and late stages of exosome maturation, changing their composition throughout this process. The lipid composition of intraluminal vesicles (ILV) in early stages is less known, but it is recognized that they are made up of cholesterol, sphingolipids, phosphatidylinositol-3-phosphate, and lysobisphosphatidic acid (LBPA) [27]. Also, this composition changes depending on the maturation stage of multivesicular bodies (MVB), as lipids have more cholesterol at early stages and LBPA in later phases [28].

When compared to donor cells, exosomes tend to be enriched in cholesterol, sphingolipids, and phosphatidylserine, and this composition differs from other EV, although their makeup depends on source-cell type [29]. Exosomes can contain ceramides, sphingomyelins, and phosphatidylcholines, all of which carry out important functions in several pathologies [30]. Concretely, exosomal ceramides were shown to act as macrophage chemoattractants in an murine model of inflammation [31]. However, another study showed that exosomes are depleted in plasma membrane lipids such as phosphatidylinositols, phosphatidylcholines, phosphatidylglycols, and phosphatidylethanolamines, whereas phosphatidylserines are present in moderate quantities in exosomes [30].

Within asthma disease, few studies have addressed exosomal lipid function. A recent article by Hough et al. described a singular lipid signature of EV in BALF obtained from asthmatics and healthy subjects [32]. They suggest that the lipid composition of EV from airways could contribute to chronic inflammation in asthma. The authors further identified characteristic differences in EV lipids between healthy subjects and asthmatics, demonstrating altered abundances in these lipids, such as ceramides, modified ceramides, and phosphatidylglycerol. Together with other lipids such as prostaglandins and leukotrienes, this lipid signature has been implicated in inflammation of asthma pathology [33,34].

Exosomes are an important source of eicosanoids (leukotrienes [LT] and prostaglandins), which have functional activity *in vivo* and *in vitro*, and they contain enzymes related to leukotriene metabolism [35]. Esser et al. demonstrated that exosomes from antigen-presenting cells (APC) such as monocyte-derived dendritic cells and macrophages contain functional enzymes implicated in LT biosynthesis [36]. The main enzymes identified in this study were leukotriene A_4 hydrolase and leukotriene C_4 synthase, which have the capacity to produce pro-inflammatory leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) from leukotriene A_4 (LTA_4). These lipid mediators are important in triggering asthma disease. Supporting these findings, Torregrosa-Paredes et al. showed that BALF-derived exosomes have an altered exosomal profile, exerting pro-inflammatory activities *in vitro* [37]. Additionally, BALF-derived exosomes contain enzymes for LT biosynthesis. These enzymes were present in exosomes from healthy and asthmatic subjects; however, only exosomes of BALF from asthmatics increased the production of LT. Additionally, co-culture of these asthmatic exosomes with bronchial epithelial cells increased LT and interleukin (IL)-8 production. Also, exosomes from lung epithelial cells carry LTC_4 that can be metabolized into LTD_4, which is the most potent mediator of bronchoconstriction [38]. In contrast, a recent study shows that exosomes from IL-1β-induced human lung fibroblasts contain prostaglandin E_2 (PGE_2) with anti-fibrotic effects [39].

2.3. Exosome-derived miRNA

MiRNA are small, non-coding RNA molecules that can be localized in several biofluids such as urine, sputum, BALF, and serum. MiRNA are loaded into exosomes during biogenesis through several strictly controlled mechanisms. Gon et al. described selective release of miRNA in exosomes [40]. They found increased levels of exosomal miRNA in BALF-derived exosomes from house-dust–stimulated mice that may inhibit Th2 molecules, such as IL-5 and IL-13. However, if the exosome release is partially blocked by GW4869 (an inhibitor of neutral sphingomyelinase), the number of exosomes in BALF is reduced and miRNA that inhibit Th2 inflammation are decreased, exacerbating eosinophilic airway inflammation.

In recent years, the number of miRNA studies has increased exponentially, as miRNA are excellent biomarkers of several diseases, including cancer and cardiovascular and respiratory diseases [41–43]. Particularly, miRNA have also been studied in asthma pathology [44]. Recently, our group published an article on eosinophil miRNA in which we report that miRNA can be used to differentiate healthy subjects from asthmatic patients [45], so this miRNA signature could be used as an asthma biomarker. This miRNA profile can distinguish healthy from asthmatics, as the two differ in the number of eosinophils and serum periostin levels. Concretely, serum miR-185-5p discriminates both groups and has the ability to classify patients according to asthma severity.

Nowadays, most studies emphasize the function of miRNA in asthma [13,46], but few address exosomal miRNA. Valadi et al. were the first to report that miRNA are present in exosomes and can be transferred to recipient cells [47]. Subsequently, several studies have shown that exosomal miRNA can participate in pathological processes such as asthma. One of these was performed by Levänen et al., who described an altered exosomal miRNA profile in BALF [48]. This miRNA set was composed of 24 miRNA expressed differentially between asthmatic and healthy subjects. These miRNA include members of the let-7 and miR-200 families. This exosomal miRNA profile can distinguish mild non-symptomatic asthmatic patients from healthy subjects. Moreover, these “exosome-shuttle miRNA” can be transferred to other cells and exert a functional effect on recipient cells. Montecalvo et al. demonstrated a novel mechanism of intercellular communication between dendritic cells (DC) based on transference of exosomal miRNA with functional activities, which suppressed target mRNA in acceptor cells [49]. This process of exosomal miRNA exchange can be one-directional, as reported in the 2011 study by Mittelbrunn et al. [50], demonstrating the unidirectional exosome-miRNA transference from T cells to APC. These findings lend support to the notion that exosomes serve as an intercellular transporter of miRNA in the lungs.

Airway remodelling is a key process in asthma disease. Gupta et al. identified a different exosomal miRNA population between two types of epithelial cells: primary tracheobronchial cells and epithelial cells derived from human lung adenocarcinoma [51]. Both cell types release a distinct set of exosomal miRNA, such as miR-34a/b/c, miR-449b/c, and miR-223. However, several miRNA associated with inflammatory and remodelling processes were present in the same levels in both types of exosomes such as miR-21, which is one of the most widely studied miRNA types in inflammatory diseases. Elbehidy et al. demonstrated that miR-21 expression was inversely correlated with serum IL-12p35 levels and forced expiratory volume in 1 s (FEV_1) in asthmatic children [52]. It is known that *IL12p35* is a target gene of miR-21, inhibiting its expression and destabilising Th1/Th2 balance to Th2 response, a characteristic of asthma disease.

3. Functions of exosomes in asthma

Exosome production has been demonstrated in almost all cell types, and this production has been found to depend on cellular cargo, on the conditions of their environment, or the stimuli that act upon these cells.

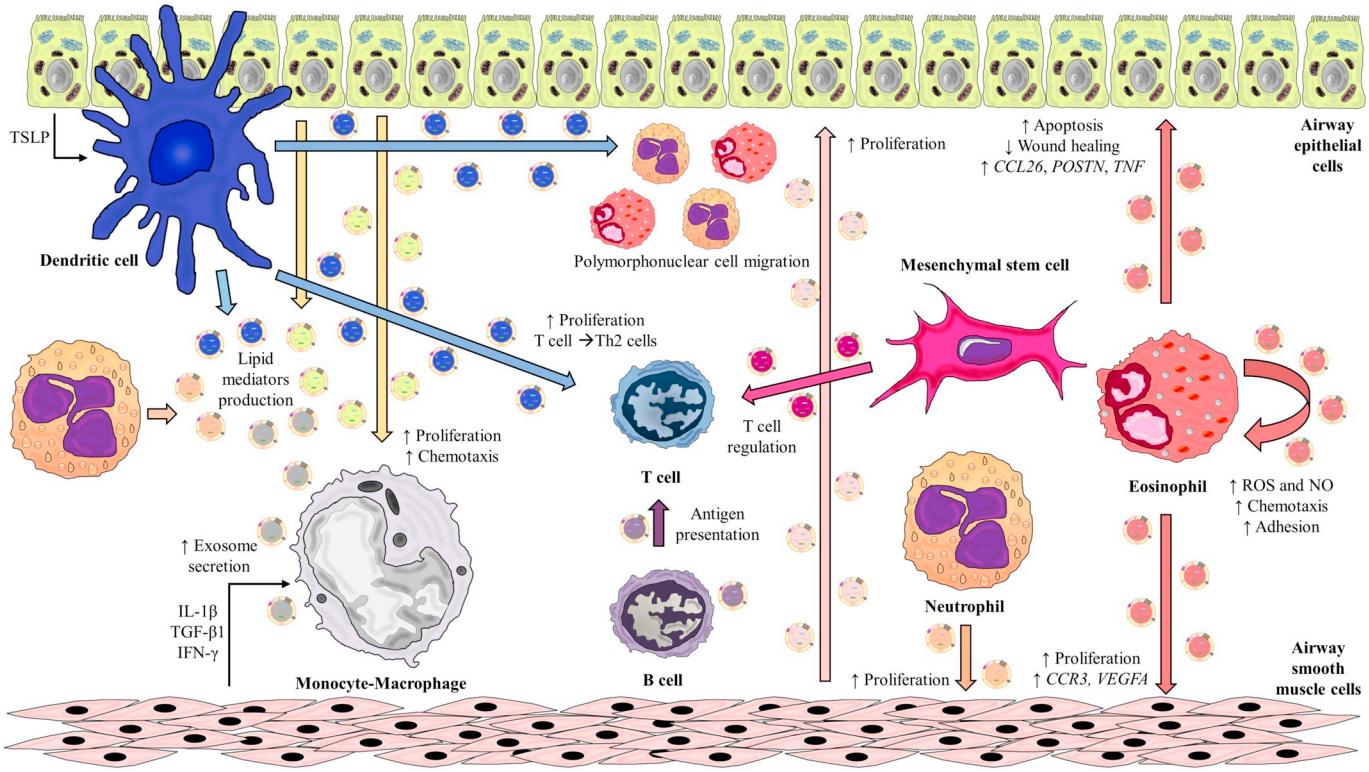

Fig. 2. Exosomes from different cellular sources can interact with the main cells involved in asthma. Most immune cells secrete exosomes, which play an important role in asthma pathogenesis. Exosomes from dendritic cells promote polymorphonuclear cell migration, and they encourage proliferation to Th2 cells from T cells. B cells produce exosomes that can act as antigen presenting units. Also, mesenchymal stem cells secrete exosomes with immunomodulatory effects over T cells. Several cytokines released from smooth muscle cells activate exosome secretion in macrophages. Neutrophil-derived exosomes stimulate smooth-muscle-mass growth and contain several enzymes to produce pro-inflammatory lipid mediators. Exosomes secreted from eosinophils have autoregulatory effects over themselves, and they act over epithelial and smooth muscle cells, increasing their proliferation, apoptosis, and gene expression of pro-inflammatory molecules. Also, structural lung cells release exosomes. Exosomes from airway epithelial cells stimulate macrophage proliferation and chemotaxis, and exosomes from smooth muscle cells increase proliferation of lung epithelial cells. IFN-γ, interferon-gamma; NO, nitric oxide; ROS, reactive oxygen species; TGF-β1, transforming growth factor beta 1; TSLP, thymic stromal lymphopoietin.

A plethora of immune cells participate in asthmatic and allergic processes, and their exosomes could play a role in the pathogenesis of these disorders. Fig. 2 summarizes the principal interactions between exosomes and major immune cells as well as the effects produced.

3.1. Exosomes from dendritic cells and monocyte-macrophages

DC usually participate in innate immunity, phagocytising pathogens to process and present their antigens to T lymphocytes. These highly specialized effector cells are considered the primary APC in the immune system and play an important role in the initiation of the immune response [53]. DC can secrete exosomes, stimulating T-cell responses through several mechanisms, either by direct contact or by means of other APC [54,55].

DC-derived exosomes and their surface components have been characterized [56]. They contain several molecules that confirm their endosomal origin, such as CD63 or CD54, the latter an adhesion molecule that allows these exosomes to interact with lymphocyte function-associated antigen (LFA)-1, a molecule present on the T-lymphocyte surface. Other molecules present in DC exosomes are major histocompatibility complex class II (MHCII), CD86, and human leukocyte antigen (HLA)-DR. Thus, DC-derived exosomes can present aeroallergens to T-cells. They trigger the production of type 2 cytokines such as IL-4, especially in allergic individuals, thereby contributing to allergic inflammation [57].

Recently, new data on the role of exosomes from DC have been reported. Huang et al. show that DC activated by thymic stromal lymphopoietin (TSLP) are able to induce the proliferation of $CD4^+$ T cells and their differentiation to Th2 cells through OX40L [58]. This finding provides a new mechanism of cell-to-cell interaction and communication.

Depending on exosome subtype and cellular status of maturation, the effect of exosomes varies. DC secrete EV of different size, protein composition, and subcellular origin, and outcomes differ based on the size and composition of T-cells [59].

Exosomes produced by monocyte-derived macrophages (MDM) are modulated by multiple factors and molecules. Several cytokines such as transforming growth factor-beta (TGF-β) 1, IL-1β, and interferon-gamma (IFN-γ) affect the rate of exosome generation and delivery by peripheral blood monocytes or alveolar macrophages [36,60,61]. Also, enzymes such as Rab guanosine triphosphatases (Rab GTPases) exert an effect over vesicular traffic at the membrane level, affecting and regulating the exosome-delivery process [62].

Exosomes from MDM and DC perform enzymatic activity that plays an essential role in inflammation. Exosomes from DC are able to induce migration and recruitment of polymorphonuclear leukocytes to the site of inflammation [36]. In addition, DC and MDM-derived exosomes participate in the arachidonic acid pathway, producing several metabolites such as LTB_4 or 5-keto eicosatetraenoic acid, which are pro-inflammatory lipid mediators that play a key role in several allergic processes, asthma pathogenesis, and chronic inflammation.

3.2. Exosomes released by lymphocytes

T and B lymphocytes carry out important functions in asthma and allergic response. They are implicated in the release of IgE against

specific antigens, mediate cytokine production, and influence the shift to Th2 phenotype in a high percentage of asthma processes. Moreover, they secrete factors that trigger numerous mechanisms such as migration of granulocytes and other pro-inflammatory effects.

Although several studies have demonstrated that T lymphocytes can release exosomes with several functions, functional studies in asthma and allergy are mainly focused on the effects that other exosome sources have on T cells [63–65]. A synthesis of studies depicts the functions of exosomes from different cellular populations with regard to T cells [12]. Recently, a manuscript published by Du et al. [66] has proven that mesenchymal stem cell (MSC) exosomes exert several effects on regulatory T cells (Treg) from healthy and asthmatic subjects. Concerning T cells, and outside of the scope of asthmatic pathology, multiple articles have shown the effect that MSC cells exert on other cell types, as in the case of DC in autoimmunity [67], while other research has shown the effect that these cells have over vascular endothelial cells, modifying their functionality and their vascular endothelial growth factor (VEGF) expression [68].

Exosomes from B cells have been studied by several research groups. One of the first manuscripts published on this topic appeared in 1996 and described how B cell-derived exosomes are able to present peptides with antigenic capacity to T cells through MHC class-II receptors [55]. Subsequent studies have demonstrated that the surface of exosomes from B cells contain a plethora of both molecules such as CD40, CD80, and CD86, which have co-stimulatory capacity as well as integrins (α1 and α2), enabling them to exert important effects over T-cell response [69–71]. More than ten years ago, Admyre et al. [72] demonstrated that B cells can act on T cells through their exosomes, triggering a potent immune response in allergic disease and highlighting the fact that exosomes can be autonomous antigen-presenting structures and do not require direct contact between cells. Similarly, serum or BALF exosomes originated by B cells or by other cell populations can exert allergic tolerogenic activity, thus giving rise to their name, “tolerosomes” [73,74].

These findings may be important for future exosome engineering aimed at creating exosome-based vaccines for immunotherapy.

3.3. Role of exosomes from eosinophils

Eosinophils are one of the key players in asthmatic pathophysiology. They are elevated in airways from asthmatics, which makes eosinophil airway count a disease biomarker [75]. In asthma pathophysiology, eosinophils secrete several proteins such as MBP, EPO, and ECP as well as other airway-damaging mediators like reactive oxygen species (ROS) or NO [75].

Our group was the first to describe that eosinophils are capable of producing MVB; when fused with plasma membrane, MVB are released into the extracellular environment as exosomes [16]. These exosomes were released in greater quantity by eosinophils from asthmatics, and contain such eosinophil-derived enzymes as EPO, MBP, and ECP regardless of whether they come from healthy or asthmatic sources. *In vitro* assays showed that exosomes from eosinophils are functional in asthma disease. Exosomes derived from the eosinophils of asthmatics act in an autocrine manner upon eosinophils themselves, a characteristic that distinguishes them from exosomes from healthy eosinophils. This type of exosomes increased ROS and NO production and augmented eosinophil chemotaxis and adhesion by upregulating ICAM-1 and integrin-α_2 [17].

As mentioned previously, eosinophils play a role in asthma by damaging the airways through secretion of a variety of enzymes and mediators [75]. We described an alternative pathway of airway-function modification by eosinophil exosomes. Small airway epithelial cells (SAEC) and bronchial smooth muscle cells (BSMC) structurally remodel the airway and perform roles in both respiratory function and the immune response. Exosomes from asthmatic eosinophils modify the behaviour of SAEC, increasing their apoptosis, reducing their wound-repair capacity, and enhancing expression of *CCL26*, *TNF*, and *POSTN*. BSMC performance is also modified by asthmatic exosomes, which increase the ERK1/2-phosphorylation-mediated proliferation of these cells and upregulate *CCR3* and *VEGFA* gene expression [18].

3.4. Role of exosomes from neutrophils

Some severe asthma phenotypes are characterized by a high number of neutrophils in the affected airways and have been associated with corticosteroid refractory asthma [76]. Neutrophils secrete cytokines like IL-6, IL-8, IL9, IL-12, and IFN-γ, orchestrating an immune response and causing damage to the airways by ROS secretion and through the action of enzymes such as metalloproteinase (MMP)-9 or elastase and DNA traps [77]. In addition, neutrophils stimulated with lipopolysaccharides secrete exosomes which are internalized by airway smooth muscle cells, enhancing their proliferation [26]. Also, in an autocrine fashion, neutrophils that migrate towards a primary chemoattractant secrete exosomes loaded with LTB_4 or its synthesizing enzymes to mediate neighbour neutrophil motility [78].

Neutrophil-platelet interactions have been studied in the context of infections, and it has been described that platelet-derived EV promote adhesion of neutrophils to endothelial cells through CD62P and CXC chemokines [79]. This communication is bidirectional, as neutrophil-derived EV are internalized in platelets, which in turn synthesize thromboxane-A2, a process which elicits endothelial expression of ICAM-1, promoting neutrophil extravasation [80].

3.5. Role of exosomes from airway structural cells

Airway structural cells, including the epithelium and smooth muscle mass, are crucial to asthma pathophysiology, and they play a key role in the defence against pathogens, mucus production, bronchoconstriction response, and airway remodelling [18,81].

Airway epithelial cells (AEC) are the principal component of the epithelium and are able to secrete exosomes. Exosomes released by AEC vary in size and shape depending on their cell-type origin and mucin content [82]. Gupta et al. showed that distinct epithelial cell types exchanged exosomes, and this transfer alters the quantitative and qualitative profile of airway secretions, including proteins and miRNA [51]. IL-13 secreted in the asthmatic immune environment also induces exosome secretion by epithelial cells, increasing chemotaxis and proliferation of macrophages [60]. Additionally, exosomes derived from lung-cancer epithelial cells contain γ-glutamyl transpeptidase 1, which catalyses LTC_4 derived from myeloid cells into LTD_4 [38]. This leukotriene is implicated in bronchoconstriction and airway remodelling.

Bronchoconstriction is a key feature in asthma, and it may trigger the positive feedback that drives the angiogenesis observed in this pathology. Bronchial epithelial cells submitted to compressive stress release exosomes that contain tissue factor, which is increased in asthmatic airways and implicated in angiogenesis [83]. Fibroblasts are also implicated in airway remodelling and also secrete exosomes. Exosomes from fibroblasts of asthmatics present lower levels of TGF-β and increase the proliferation of epithelial cells [84].

4. Exosomes as therapeutic tools

Exosomes are an important tool for diagnosis. Also, they are used as biomarkers in several diseases and have therapeutic applications [85,86]. Due to their molecular characteristics, different approaches are being developed in the field of exosome therapy, principally as a drug-delivery vehicle. Currently, the most favoured drug-delivery systems are polymeric nanoparticles and liposomes [87,88]. These carriers have been used to deliver different therapeutic molecules and drugs; however, they have several disadvantages: (i) low stability, (ii) inability to evade the host immune system, and (iii) potential toxicity [89]. To resolve these issues, exosomes can be chosen as drug-delivery vesicles

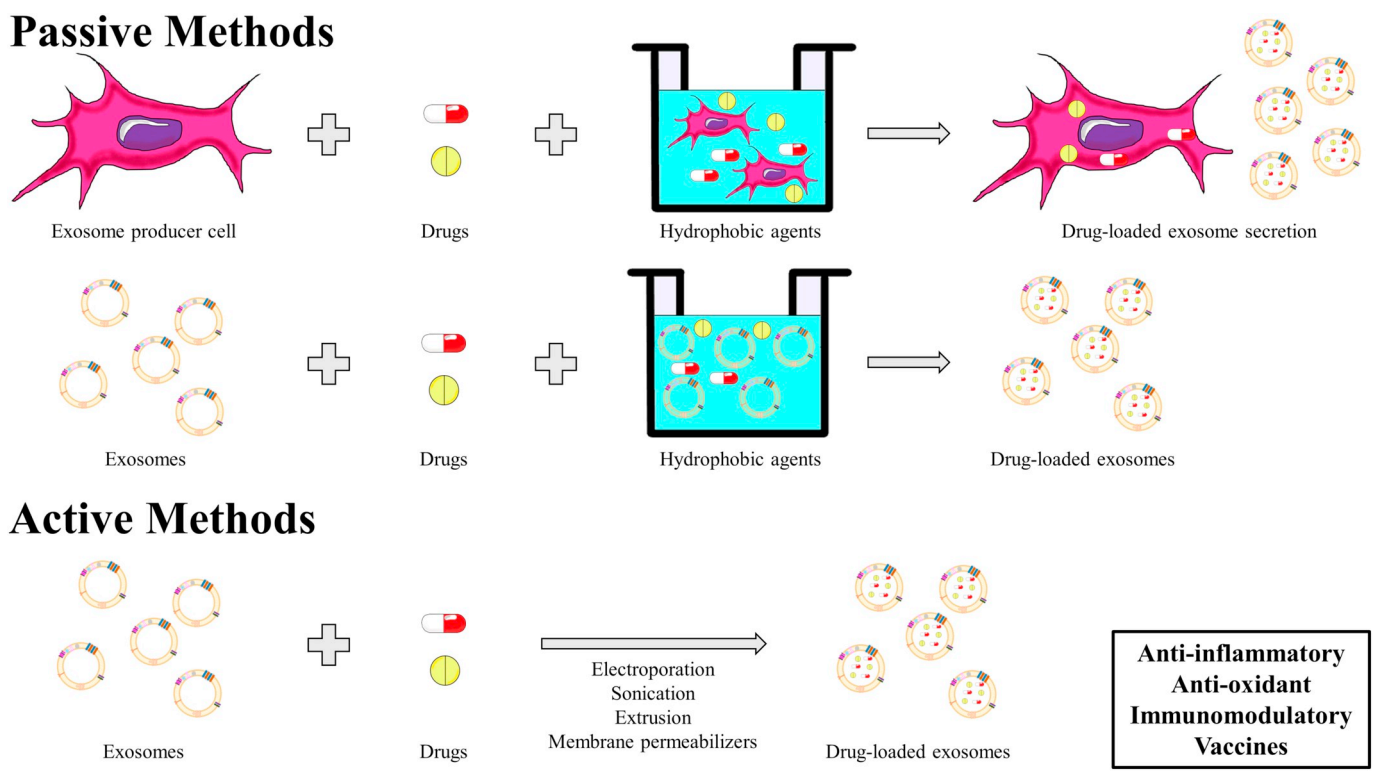

Fig. 3. Drug-loaded exosome methods. Exosomes can be employed as therapeutic tools. There are two distinct methods of loading molecular cargo into exosomes: passive and active techniques. Passive methods involve the use of hydrophobic agents, which are incubated with exosomes or exosome-releasing donor cells. Active methods are based on the use of extrusion, sonication, or electroporation techniques to destabilize exosomal-membrane integrity, allowing drugs or other molecules to be introduced into exosomes and having anti-inflammatory, anti-oxidant, and immunomodulatory effects. Also, these drug-loaded exosomes can be used as vaccines in several diseases.

because as particles they are degradation-resistant and more stable, they produce low immune responses, cross the blood-brain barrier, have non-tumorigenic properties, and transport different molecules such as small molecules (curcumin) [90], proteins, and enzymes (catalase and superoxide dismutase) [91] just like nucleic acids (siRNA, miRNA) [92].

Several strategic approaches have been developed to load molecular cargo into exosomes, which are classified into two major groups: passive and active [93]. The former employs techniques with hydrophobic agents, which are incubated with exosomes or donor cells that release exosomes [94]. Active methods are based on techniques such as extrusion, sonication, electroporation, freeze and thaw cycles, and use of membrane permeabilizers enabling molecules and drugs to be introduced into exosomes by destabilising exosomal-membrane integrity [93]. Fig. 3 outlines the different methods of loading therapeutic molecules and drugs into exosomes.

A variety of studies explore exosomes as therapeutic tools for inflammatory diseases, but only a few have been conducted in asthma. One of the first studies was performed in 2008 by Almqvist et al. [73]. In this study, the authors used a murine model to investigate allergic asthma, determining whether serum-derived exosomes from OVA-fed donors (also called “tolerosomes”) protect the lungs against airway inflammation. They demonstrated that mice that have received “tolerosomes” present reduced levels of both the total number of cells and eosinophils in BALF. In another use, the exosomes from MSC are widely used for therapeutic approaches. Several studies have demonstrated that MSC can alleviate airway inflammation in asthma. In 2017, Du et al. showed that MSC exosomes increase Treg percentage in peripheral blood mononuclear cells (PBMC), heighten proliferation, and promote immunosuppressive capacity by upregulating anti-inflammatory cytokines (IL-10 and TGF-β1) in PBMC [66]. Recently, Cho et al. have demonstrated that exosomes from adipose tissue-derived MSC have a potential therapeutic effect on an atopic-dermatitis mouse model [95]. These exosomes downregulated mRNA levels of IL-4, IL-23, IL-31, and tumor necrosis factor alpha (TNF-α) in skin lesions.

Exosomes can serve as drug-delivery vehicles and vaccines, and they carry different therapeutic drugs to treat a number of diseases. One of these drugs is curcumin, which has anti-oxidant, immunomodulatory, and anti-inflammatory (reducing pro-inflammatory cytokines) effects in several diseases [96]. Exosomes may also serve as vaccines. For example, several research groups from the French Gustave Roussy Institute and Curie Institute have completed a phase I clinical trial on vaccines based on the exosomes of dendritic cells (also called “dexosomes”) against metastatic melanoma [97]. To date, however, there have been no exosome-based vaccine studies in asthma.

Despite such potential, important barriers remain, impeding the immediate clinical use of exosomes as a therapy [98]. First, the exact mechanism by which exosomes cross the blood-brain barrier is not fully understood. Second, the exact molecular cargo of exosomes has yet to be determined, because some of these molecules could be implicated in pathological mechanisms. Last, exosome isolation and the loading of cargo into exosomes remain costly and inefficient techniques. Therefore, more studies are needed to develop effective exosome-based therapy.

5. Conclusions

In this review, we have focused on recent advances in the field of exosomes and asthma pathology. Exosomes have a complex molecular composition, and their cargo is extremely heterogeneous, with variation stemming from their cellular origin and physiological state. Exosomes play an important role in multiple diseases and have been widely studied. However, their specific functions in asthma are much less understood, although over recent years there has been an increase

in the number of studies of exosomes in this pathology context. It is well known that almost all types of inflammatory cells release exosomes, which in turn exert a number of effects associated with asthma disease. Moreover, exosomes have been found in biofluids such as BALF, which influences various cell types and modifies processes associated with asthma.

Exosomes hold potential as tools for the diagnosis and treatment of several diseases, particularly in asthma. These nanovesicles contain important molecules implicated in the pathophysiologic mechanism of multiple disorders, and by means of molecular engineering their composition can be modified and therapeutic drugs can be loaded into them.

Despite the progress made, more studies are necessary to arrive at a deep knowledge of how exosomes act in asthma disease and develop effective treatments based on this knowledge.

Conflict of interest

All authors declare that none presents a conflict of interest.

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References

[1] T. To, S. Stanojevic, G. Moores, A.S. Gershon, E.D. Bateman, A.A. Cruz, L.P. Boulet, Global asthma prevalence in adults: findings from the cross-sectional world health survey, BMC Public Health 12 (2012) 204, , https://doi.org/10.1186/1471-2458-12-204.

[2] K.F. Chung, S.E. Wenzel, J.L. Brozek, A. Bush, M. Castro, P.J. Sterk, I.M. Adcock, E.D. Bateman, E.H. Bel, E.R. Bleecker, L.P. Boulet, C. Brightling, P. Chanez, S.E. Dahlen, R. Djukanovic, U. Frey, M. Gaga, P. Gibson, Q. Hamid, N.N. Jajour, T. Mauad, R.L. Sorkness, W.G. Teague, International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma, Eur. Respir. J. 43 (2) (2014) 343–373, https://doi.org/10.1183/09031936.00202013.

[3] S.T. Holgate, S. Wenzel, D.S. Postma, S.T. Weiss, H. Renz, P.D. Sly, Asthma, Nat. Rev. Dis. Prim. 1 (2015) 15025, , https://doi.org/10.1038/nrdp.2015.25.

[4] G. Pelaia, A. Vatrella, M.T. Busceti, L. Gallelli, C. Calabrese, R. Terracciano, R. Maselli, Cellular mechanisms underlying eosinophilic and neutrophilic airway inflammation in asthma, Mediat. Inflamm. 2015 (879783) (2015), https://doi.org/10.1155/2015/879783.

[5] A. Ray, T.B. Oriss, S.E. Wenzel, Emerging molecular phenotypes of asthma, Am. J. Physiol. Cell. Mol. Physiol. 308 (2) (2015) L130–L140, https://doi.org/10.1152/ajplung.00070.2014.

[6] M. Gauthier, A. Ray, S.E. Wenzel, Evolving concepts of asthma, Am. J. Respir. Crit. Care Med. 192 (6) (2015) 660–668, https://doi.org/10.1164/rccm.201504-0763PP.

[7] J.R. Stokes, T.B. Casale, Characterization of asthma endotypes: implications for therapy, Ann. Allergy Asthma Immunol. 117 (2) (2016) 121–125, https://doi.org/10.1016/j.anai.2016.05.016.

[8] A. Lo Cicero, P.D. Stahl, G. Raposo, Extracellular vesicles shuffling intercellular messages: for good or for bad, Curr. Opin. Cell Biol. 35 (2015) 69–77, https://doi.org/10.1016/j.ceb.2015.04.013.

[9] B. Dörsam, K.S. Reiners, E.P. von Strandmann, Cancer-derived extracellular vesicles: friend and foe of tumour immunosurveillance, Philos. Trans. R. Soc. B Biol. Sci. 373 (1737) (2015) 20160481, , https://doi.org/10.1098/rstb.2016.0481.

[10] J.S. Schorey, Y. Cheng, P.P. Singh, V.L. Smith, Exosomes and other extracellular vesicles in host-pathogen interactions, EMBO Rep. 16 (1) (2015) 24–43, https://doi.org/10.15252/embr.201439363.

[11] Y. Zhang, Y.W. Hu, L. Zheng, Q. Wang, Characteristics and roles of exosomes in cardiovascular disease, DNA Cell Biol. 36 (3) (2017) 202–211, https://doi.org/10.1089/dna.2016.3496.

[12] K.P. Hough, D. Chanda, S.R. Duncan, V.J. Thannickal, J.S. Deshane, Exosomes in immunoregulation of chronic lung diseases, Allergy 72 (4) (2017) 534–544, https://doi.org/10.1111/all.13086.

[13] B. Sastre, J.A. Cañas, J.M. Rodrigo-Muñoz, V. del Pozo, Novel modulators of asthma and allergy: exosomes and MicroRNAs, Front. Immunol. 8 (2017) 826, , https://doi.org/10.3389/fimmu.2017.00826.

[14] E. Mortaz, S.D. Alipoor, M. Varahram, H. Jamaati, J. Garssen, S.E. Mumby, I.M. Adcock, Exosomes in severe asthma: update in their roles and potential in therapy, Biomed. Res. Int. 2018 (2018) 2862187, , https://doi.org/10.1155/2018/2862187.

[15] C.J.E. Wahlund, A. Eklund, J. Grunewald, S. Gabrielsson, Pulmonary extracellular vesicles as mediators of local and systemic inflammation, Front. Cell Dev. Biol. 5 (2017) 39, , https://doi.org/10.3389/fcell.2017.00039.

[16] C. Mazzeo, J.A. Cañas, M.P. Zafra, A. Rojas Marco, M. Fernández-Nieto, V. Sanz, M. Mittelbrunn, M. Izquierdo, F. Baixaulli, J. Sastre, V. del Pozo, Exosome secretion by eosinophils: a possible role in asthma pathogenesis, J. Allergy Clin. Immunol. 135 (6) (2015) 1603–1613, https://doi.org/10.1016/j.jaci.2014.11.026.

[17] J.A. Cañas, B. Sastre, C. Mazzeo, M. Fernández-Nieto, J.M. Rodrigo-Muñoz, A. González-Guerra, M. Izquierdo, P. Barranco, S. Quirce, J. Sastre, V. del Pozo, Exosomes from eosinophils autoregulate and promote eosinophil functions, J. Leukoc. Biol. 101 (5) (2017) 1191–1199, https://doi.org/10.1189/jlb.3AB0516-233RR.

[18] J.A. Cañas, B. Sastre, J.M. Rodrigo-Muñoz, M. Fernández-Nieto, P. Barranco, S. Quirce, J. Sastre, V. del Pozo, Eosinophil-derived exosomes contribute to asthma remodelling by activating structural lung cells, Clin. Exp. Allergy 48 (9) (2018) 1173–1185, https://doi.org/10.1111/cea.13122.

[19] C. Théry, M. Ostrowski, E. Segura, Membrane vesicles as conveyors of immune responses, Nat. Rev. Immunol. 9 (8) (2009) 581–593, https://doi.org/10.1038/nri2567.

[20] M. Pathan, P. Fonseka, S.V. Chitti, T. Kang, R. Sanwlani, J. Van Deun, A. Hendrix, S. Mathivanan, Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles, Nucleic Acids Res. (2018), https://doi.org/10.1093/nar/gky1029 In press.

[21] S. Keerthikumar, D. Chisanga, D. Ariyaratne, H. Al Saffar, S. Anand, K. Zhao, M. Samuel, M. Pathan, M. Jois, N. Chilamkurti, L. Gangoda, S. Mathivanan, ExoCarta: a web-based compendium of exosomal cargo, J. Mol. Biol. 428 (4) (2016) 688–692, https://doi.org/10.1016/j.jmb.2015.09.019.

[22] V. Rollet-Cohen, M. Bourderioux, J. Lipecka, C. Chhuon, V.A. Jung, M. Mesbahi, T. Nguyen-Khoa, S. Guérin-Pfyffer, A. Schmitt, A. Edelman, I. Sermet-Gaudelus, I.C. Guerrera, Comparative proteomics of respiratory exosomes in cystic fibrosis, primary ciliary dyskinesia and asthma, J. Proteome 185 (2018) 1–7, https://doi.org/10.1016/j.jprot.2018.07.001.

[23] C. Lässer, S.E. O'Neil, G.V. Shelke, C. Sihlbom, S.F. Hansson, Y.S. Gho, B. Lundbäck, J. Lötvall, Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation, J. Transl. Med. 14 (2016) 181, , https://doi.org/10.1186/s12967-016-0927-4.

[24] E.I. Buzás, E.Á. Tóth, B.W. Sódar, K.É. Szabó-Taylor, Molecular interactions at the surface of extracellular vesicles, Semin. Immunopathol. 40 (5) (2018) 453–464, https://doi.org/10.1007/s00281-018-0682-0.

[25] K.C. French, M.A. Antonyak, R.A. Cerione, Extracellular vesicle docking at the cellular port: extracellular vesicle binding and uptake, Semin. Cell Dev. Biol. 67 (2017) 48–55, https://doi.org/10.1016/j.semcdb.2017.01.002.

[26] A. Vargas, F. Roux-Dalvai, A. Droit, J.P. Lavoie, Neutrophil-derived exosomes: a new mechanism contributing to airway smooth muscle remodeling, Am. J. Respir. Cell Mol. Biol. 55 (3) (2016) 450–461, https://doi.org/10.1165/rcmb.2016-0033OC.

[27] J. Huotari, A. Helenius, Endosome maturation, EMBO J. 30 (17) (2011) 3481–3500, https://doi.org/10.1038/emboj.2011.286.

[28] W. Möbius, E. van Donselaar, Y. Ohno-Iwashita, Y. Shimada, H.F.G. Heijnen, J.W. Slot, H.J. Geuze, Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway, Traffic 4 (4) (2003) 222–231, https://doi.org/10.1034/j.1600-0854.2003.00072.x.

[29] T. Skotland, N.P. Hessvik, K. Sandvig, A. Llorente, Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology, J. Lipid Res. 60 (1) (2019) 9–18, https://doi.org/10.1194/jlr.R084343.

[30] R.A. Haraszti, M.C. Didiot, E. Sapp, J. Leszyk, S.A. Shaffer, H.E. Rockwell, F. Gao, N.R. Narain, M. DiFiglia, M.A. Kiebish, N. Aronin, A. Khvorova, High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources, J. Extracell. Vesicles 5 (2016) 32570, , https://doi.org/10.3402/jev.v5.32570.

[31] E. Kakazu, A.S. Mauer, M. Yin, H. Malhi, Hepatocytes release ceramide-enriched pro-inflammatory extracellular vesicles in an IRE1α-dependent manner, J. Lipid Res. 57 (2) (2016) 233–245, https://doi.org/10.1194/jlr.M063412.

[32] K.P. Hough, L.S. Wilson, J.L. Trevor, J.G. Strenkowski, N. Maina, Y.I. Kim, M.L. Spell, Y. Wang, D. Chanda, J.R. Dager, N.S. Sharma, M. Curtiss, V.B. Antony, M.T. Dransfield, D.D. Chaplin, C. Steele, S. Barnes, S.R. Duncan, J.K. Prasain, V.J. Thannickal, J.S. Deshane, Unique lipid signatures of extracellular vesicles from the airways of asthmatics, Sci. Rep. 8 (1) (2018) 10340, , https://doi.org/10.1038/s41598-018-28655-9.

[33] J.G. Ono, T.S. Worgall, S. Worgall, Airway reactivity and sphingolipids-implications for childhood asthma, Mol. Cell. Pediatr. 2 (1) (2015) 13, https://doi.org/10.1186/s40348-015-0025-3.

[34] E. Schauberger, M. Peinhaupt, T. Cazares, A.W. Lindsley, Lipid mediators of allergic disease: pathways, treatments, and emerging therapeutic targets, Curr Allergy Asthma Rep 16 (7) (2016) 48, https://doi.org/10.1007/s11882-016-0628-3.

[35] M. Record, K. Carayon, M. Poirot, S. Silvente-Poirot, Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies, Biochim. Biophys. Acta 1841 (1) (2014) 108–120, https://doi.org/10.1016/j.bbalip.2013.10.004.

[36] J. Esser, U. Gehrmann, F.L. D'Alexandri, A.M. Hidalgo-Estévez, C.E. Wheelock,

A. Scheynius, S. Gabrielsson, O. Rådmark, Exosomes from human macrophages and dendritic cells contain enzymes for leukotriene biosynthesis and promote granulocyte migration, J. Allergy Clin. Immunol. 126 (5) (2010) 1032–1040, https://doi.org/10.1016/j.jaci.2010.06.039.
[37] P. Torregrosa Paredes, J. Esser, C. Admyre, M. Nord, Q.K. Rahman, A. Lukic, O. Rådmark, R. Grönneberg, J. Grunewald, A. Eklund, A. Scheynius, S. Gabrielsson, Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma, Allergy 67 (7) (2012) 911–919, https://doi.org/10.1111/j.1398-9995.2012.02835.x.
[38] A. Lukic, J. Ji, H. Idborg, B. Samuelsson, L. Palmberg, S. Gabrielsson, O. Rådmark, Pulmonary epithelial cancer cells and their exosomes metabolize myeloid cell-derived leukotriene C4 to leukotriene D4, J. Lipid Res. 57 (9) (2016) 1659–1669, https://doi.org/10.1194/jlr.M066910.
[39] S.H. Lacy, C.F. Woeller, T.H. Thatcher, S.J. Pollock, E.M. Small, P.J. Sime, R.P. Phipps, Activated human lung fibroblasts produce extracellular vesicles with anti-fibrotic prostaglandins, Am. J. Respir. Cell Mol. Biol. (2018), https://doi.org/10.1165/rcmb.2017-0248OC In press.
[40] Y. Gon, S. Maruoka, T. Inoue, K. Kuroda, K. Yamagishi, Y. Kozu, S. Shikano, K. Soda, J. Lötvall, S. Hashimoto, Selective release of miRNAs via extracellular vesicles is associated with house-dust mite allergen-induced airway inflammation, Clin. Exp. Allergy 47 (12) (2017) 1586–1598, https://doi.org/10.1111/cea.13016.
[41] R. Munagala, F. Aqil, R.C. Gupta, Exosomal miRNAs as biomarkers of recurrent lung cancer, Tumor Biol. 37 (8) (2016) 10703–10714, https://doi.org/10.1007/s13277-016-4939-8.
[42] R. Navickas, D. Gal, A. Laucevičius, A. Taparauskaitė, M. Zdanytė, P. Holvoet, Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systematic review, Cardiovasc. Res. 111 (4) (2016) 322–337, https://doi.org/10.1093/cvr/cvw174.
[43] S.D. Alipoor, I.M. Adcock, J. Garssen, E. Mortaz, M. Varahram, M. Mirsaeidi, A. Velayati, The roles of miRNAs as potential biomarkers in lung diseases, Eur. J. Pharmacol. 791 (2016) 395–404, https://doi.org/10.1016/j.ejphar.2016.09.015.
[44] E. Heffler, A. Allegra, G. Pioggia, G. Picardi, C. Musolino, S. Gangemi, MicroRNA profiling in asthma: potential biomarkers and therapeutic targets, Am. J. Respir. Cell Mol. Biol. 57 (6) (2017) 642–650, https://doi.org/10.1165/rcmb.2016-0231TR.
[45] J.M. Rodrigo-Muñoz, J.A. Cañas, B. Sastre, N. Rego, G. Greif, M. Rial, P. Mínguez, I. Mahíllo-Fernández, M. Fernández-Nieto, I. Mora, P. Barranco, S. Quirce, J. Sastre, V. del Pozo, Asthma diagnosis using integrated analysis of eosinophil microRNAs, Allergy (2018), https://doi.org/10.1111/all.13570 In press.
[46] M. van den Berge, H. Tasena, Role of microRNAs and exosomes in asthma, Curr. Opin. Pulm. Med. 25 (1) (2019) 87–93, https://doi.org/10.1097/MCP.0000000000000532.
[47] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J.J. Lee, J.O. Lötvall, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells, Nat. Cell Biol. 9 (6) (2007) 654–659, https://doi.org/10.1038/ncb1596.
[48] B. Levänen, N.R. Bhakta, P. Torregrosa Paredes, R. Barbeau, S. Hiltbrunner, J.L. Pollack, C.M. Sköld, M. Svartengren, J. Grunewald, S. Gabrielsson, A. Eklund, B.M. Larsson, P.G. Woodruff, D.J. Erle, Å.M. Wheelock, Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients, J. Allergy Clin. Immunol. 131 (3) (2013) 894–903, https://doi.org/10.1016/j.jaci.2012.11.039.
[49] A. Montecalvo, A.T. Larregina, W.J. Shufesky, D.B. Stolz, M.L.G. Sullivan, J.M. Karlsson, C.J. Baty, G.A. Gibson, G. Erdos, Z. Wang, J. Milosevic, O.A. Tkacheva, S.J. Divito, R. Jordan, J. Lyons-Weiler, S.C. Watkins, A.E. Morelli, Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes, Blood 119 (3) (2012) 756–766, https://doi.org/10.1182/blood-2011-02-338004.
[50] M. Mittelbrunn, C. Gutiérrez-Vázquez, C. Villarroya-Beltri, S. González, F. Sánchez-Cabo, M.Á. González, A. Bernad, F. Sánchez-Madrid, Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells, Nat. Commun. 2 (2011) 282, , https://doi.org/10.1038/ncomms1285.
[51] R. Gupta, G. Radicioni, S. Abdelwahab, H. Dang, J. Carpenter, M. Chua, P.A. Mieczkowski, J. Sheridan, S.H. Randell, M. Kesimer, Intercellular communication between airway epithelial cells is mediated by exosome-like vesicles, Am. J. Respir. Cell Mol. Biol. (2018), https://doi.org/10.1165/rcmb.2018-0156OC In press.
[52] R.M. Elbehidy, D.M. Youssef, A.S. El-Shal, S.M. Shalaby, H.S. Sherbiny, L.M. Sherief, N.E. Akeel, MicroRNA-21 as a novel biomarker in diagnosis and response to therapy in asthmatic children, Mol. Immunol. 71 (2016) 107–114, https://doi.org/10.1016/j.molimm.2015.12.015.
[53] L. Zitvogel, J.I. Mayordomo, T. Tjandrawan, A.B. DeLeo, M.R. Clarke, M.T. Lotze, W.J. Storkus, Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines, J. Exp. Med. 183 (1) (1996) 87–97, https://doi.org/10.1084/jem.183.1.87.
[54] C. Théry, L. Duban, E. Segura, P. Véron, O. Lantz, S. Amigorena, Indirect activation of naïve CD4+ T cells by dendritic cell-derived exosomes, Nat. Immunol. 3 (12) (2002) 1156–1162, https://doi.org/10.1038/ni854.
[55] G. Raposo, H.W. Nijman, W. Stoorvogel, R. Liejendekker, C.V. Harding, C.J. Melief, H.J. Geuze, B lymphocytes secrete antigen-presenting vesicles, J. Exp. Med. 183 (3) (1996) 1161–1172, https://doi.org/10.1084/jem.183.3.1161.
[56] C. Admyre, J. Grunewald, J. Thyberg, S. Gripenbäck, G. Tornling, A. Eklund, A. Scheynius, S. Gabrielsson, Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid, Eur. Respir. J. 22 (4) (2003) 578–583, https://doi.org/10.1183/09031936.03.00041703.
[57] H. Vallhov, C. Gutzeit, K. Hultenby, R. Valenta, H. Grönlund, A. Scheynius, Dendritic cell-derived exosomes carry the major cat allergen Fel d 1 and induce an allergic immune response, Allergy 70 (12) (2015) 1651–1655, https://doi.org/10.1111/all.12701.
[58] L. Huang, X. Zhang, M. Wang, Z. Chen, Y. Yan, W. Gu, J. Tan, W. Jiang, W. Ji, Exosomes from thymic stromal lymphopoietin-activated dendritic cells promote Th2 differentiation through the OX40 ligand, Pathobiology. (2018), https://doi.org/10.1159/000493013 (In press).
[59] M. Tkach, J. Kowal, A.E. Zucchetti, L. Enserink, M. Jouve, D. Lankar, M. Saitakis, L. Martin-Jaular, C. Théry, Qualitative differences in T-cell activation by dendritic cell-derived extracellular vesicle subtypes, EMBO J. 36 (20) (2017) 3012–3028, https://doi.org/10.15252/embj.201696003.
[60] A. Kulshreshtha, T. Ahmad, A. Agrawal, B. Ghosh, Proinflammatory role of epithelial cell-derived exosomes in allergic airway inflammation, J. Allergy Clin. Immunol. 131 (4) (2013) 1194–1203, https://doi.org/10.1016/j.jaci.2012.12.1565.
[61] N. Ismail, Y. Wang, D. Dakhlallah, L. Moldovan, K. Agarwal, K. Batte, P. Shah, J. Wisler, T.D. Eubank, S. Tridandapani, M.E. Paulaitis, M.G. Piper, C.B. Marsh, Macrophage microvesicles induce macrophage differentiation and miR-223 transfer, Blood 121 (6) (2013) 984–995, https://doi.org/10.1182/blood-2011-08-374793.
[62] M. Ostrowski, N.B. Carmo, S. Krumeich, I. Fanget, G. Raposo, A. Savina, C.F. Moita, K. Schauer, A.N. Hume, R.P. Freitas, B. Goud, P. Benaroch, N. Hacohen, M. Fukuda, C. Desnos, M.C. Seabra, F. Darchen, S. Amigorena, L.F. Moita, C. Thery, Rab27a and Rab27b control different steps of the exosome secretion pathway, Nat. Cell Biol. 12 (1) (2010) 19–30, https://doi.org/10.1038/ncb2000.
[63] P.J. Peters, H.J. Geuze, H.A. Van der Donk, J.W. Slot, J.M. Griffith, N.J. Stam, H.C. Clevers, J. Borst, Molecules relevant for T cell-target cell interaction are present in cytolytic granules of human T lymphocytes, Eur. J. Immunol. 19 (8) (1989) 1469–1475, https://doi.org/10.1002/eji.1830190819.
[64] P.J. Peters, J. Borst, V. Oorschot, M. Fukuda, O. Krähenbühl, J. Tschopp, J.W. Slot, H.J. Geuze, Cytotoxic T lymphocyte granules are secretory lysosomes, containing both perforin and granzymes, J. Exp. Med. 173 (5) (1991) 1099–1109, https://doi.org/10.1084/jem.173.5.1099.
[65] N. Blanchard, D. Lankar, F. Faure, A. Regnault, C. Dumont, G. Raposo, C. Hivroz, TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex, J. Immunol. 168 (7) (2002) 3235–3241, https://doi.org/10.4049/jimmunol.168.7.3235.
[66] Y.M. Du, Y.X. Zhuansun, R. Chen, L. Lin, Y. Lin, J.G. Li, Mesenchymal stem cell exosomes promote immunosuppression of regulatory T cells in asthma, Exp. Cell Res. 363 (1) (2018) 114–120, https://doi.org/10.1016/j.yexcr.2017.12.021.
[67] S.L. Tung, D.A. Boardman, M. Sen, M. Letizia, Q. Peng, N. Cianci, L. Dioni, L.M. Carlin, R. Lechler, V. Bollati, G. Lombardi, L.A. Smyth, Regulatory T cell-derived extracellular vesicles modify dendritic cell function, Sci. Rep. 8 (1) (2018) 6065, https://doi.org/10.1038/s41598-018-24531-8.
[68] S. Kaur, S.P. Singh, A.G. Elkahloun, W. Wu, M.S. Abu-Asab, D.D. Roberts, CD47-dependent immunomodulatory and angiogenic activities of extracellular vesicles produced by T cells, Matrix Biol. 37 (2014) 49–59, https://doi.org/10.1016/j.matbio.2014.05.007.
[69] S.I. Buschow, B.W.M. van Balkom, M. Aalberts, A.J.R. Heck, M. Wauben, W. Stoorvogel, MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis, Immunol. Cell Biol. 88 (8) (2010) 851–856, https://doi.org/10.1038/icb.2010.64.
[70] A. Muntasell, A.C. Berger, P.A. Roche, T cell-induced secretion of MHC class II-peptide complexes on B cell exosomes, EMBO J. 26 (19) (2007) 4263–4272, https://doi.org/10.1038/sj.emboj.7601842.
[71] A. Clayton, A. Turkes, S. Dewitt, R. Steadman, M.D. Mason, M.B. Hallett, Adhesion and signaling by B cell-derived exosomes: the role of integrins, FASEB J. 18 (9) (2004) 977–979, https://doi.org/10.1096/fj.03-1094fje.
[72] C. Admyre, B. Bohle, S.M. Johansson, M. Focke-Tejkl, R. Valenta, A. Scheynius, S. Gabrielsson, B cell-derived exosomes can present allergen peptides and activate allergen-specific T cells to proliferate and produce TH2-like cytokines, J. Allergy Clin. Immunol. 120 (6) (2007) 1418–1424, https://doi.org/10.1016/j.jaci.2007.06.040.
[73] N. Almqvist, A. Lönnqvist, S. Hultkrantz, C. Rask, E. Telemo, Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma, Immunology 125 (1) (2008) 21–27, https://doi.org/10.1111/j.1365-2567.2008.02812.x.
[74] N. Prado, E.G. Marazuela, E. Segura, H. Fernández-García, M. Villalba, C. Théry, R. Rodríguez, E. Batanero, Exosomes from bronchoalveolar fluid of tolerized mice prevent allergic reaction, J. Immunol. 181 (2) (2008) 1519–1525, https://doi.org/10.4049/jimmunol.181.2.1519.
[75] B. Sastre, J. Rodrigo-Muñoz, D. Garcia-Sanchez, J. Cañas, V. del Pozo, Eosinophils: old players in a new game, J Investig Allergol Clin Immunol 28 (5) (2018) 289–304, https://doi.org/10.18176/jiaci.0295.
[76] A. Ray, J.K. Kolls, Neutrophilic inflammation in asthma and association with disease severity, Trends Immunol. 38 (12) (2017) 942–954, https://doi.org/10.1016/j.it.2017.07.003.
[77] O. Ciepiela, M. Ostafin, U. Demkow, Neutrophils in asthma—a review, Respir. Physiol. Neurobiol. 209 (2015) 13–16, https://doi.org/10.1016/j.resp.2014.12.004.
[78] R. Majumdar, A. Tavakoli Tameh, C.A. Parent, Exosomes mediate LTB4 release during neutrophil chemotaxis, PLoS Biol. 14 (1) (2016) e1002336, , https://doi.org/10.1371/journal.pbio.1002336.
[79] S.J. Kuravi, P. Harrison, G.E. Rainger, G.B. Nash, Ability of platelet-derived extracellular vesicles to promote neutrophil-endothelial cell interactions, Inflammation (2018), https://doi.org/10.1007/s10753-018-0893-5 (In press).
[80] J. Rossaint, K. Kühne, J. Skupski, H. Van Aken, M.R. Looney, A. Hidalgo,

A. Zarbock, Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response, Nat. Commun. 7 (2016) 13464, , https://doi.org/10.1038/ncomms13464.
[81] J.L. Cho, M.F. Ling, D.C. Adams, L. Faustino, S.A. Islam, R. Afshar, J.W. Griffith, R.S. Harris, A. Ng, G. Radicioni, A.A. Ford, A.K. Han, R. Xavier, W.W. Kwok, R. Boucher, J.J. Moon, D.L. Hamilos, M. Kesimer, M.J. Suter, B.D. Medoff, A.D. Luster, Allergic asthma is distinguished by sensitivity of allergen-specific CD4+ T cells and airway structural cells to type 2 inflammation, Sci. Transl. Med. 8 (359) (2016) 359ra132, https://doi.org/10.1126/scitranslmed.aag1370.
[82] M. Kesimer, R. Gupta, Physical characterization and profiling of airway epithelial derived exosomes using light scattering, Methods 87 (2015) 59–63, https://doi.org/10.1016/j.ymeth.2015.03.013.
[83] J.A. Park, A.S. Sharif, D.J. Tschumperlin, L. Lau, R. Limbrey, P. Howarth, J.M. Drazen, Tissue factor-bearing exosome secretion from human mechanically stimulated bronchial epithelial cells in vitro and in vivo, J. Allergy Clin. Immunol. 130 (6) (2012) 1375–1383, https://doi.org/10.1016/j.jaci.2012.05.031.
[84] I. Haj-Salem, S. Plante, A.S. Gounni, M. Rouabhia, J. Chakir, Fibroblast-derived exosomes promote epithelial cell proliferation through TGF-β2 signalling pathway in severe asthma, Allergy 73 (1) (2018) 178–186, https://doi.org/10.1111/all.13234.
[85] J. Salimian, H. Mirzaei, A. Moridikia, A. Harchegani, A. Sahebkar, H. Salehi, Chronic obstructive pulmonary disease: MicroRNAs and exosomes as new diagnostic and therapeutic biomarkers, J. Res. Med. Sci. 23 (2018) 27, https://doi.org/10.4103/jrms.JRMS_1054_17.
[86] M. Garcia-Contreras, R.W. Brooks, L. Boccuzzi, P.D. Robbins, C. Ricordi, Exosomes as biomarkers and therapeutic tools for type 1 diabetes mellitus, Eur. Rev. Med. Pharmacol. Sci. 21 (12) (2017) 2940–2956.
[87] U. Agrawal, R. Sharma, M. Gupta, S.P. Vyas, Is nanotechnology a boon for oral drug delivery? Drug Discov. Today 19 (10) (2014) 1530–1546, https://doi.org/10.1016/j.drudis.2014.04.011.
[88] L. Sercombe, T. Veerati, F. Moheimani, S.Y. Wu, A.K. Sood, S. Hua, Advances and challenges of liposome assisted drug delivery, Front. Pharmacol. 6 (2015) 286, , https://doi.org/10.3389/fphar.2015.00286.
[89] K. Raemdonck, K. Braeckmans, J. Demeester, S.C. De Smedt, Merging the best of both worlds: hybrid lipid-enveloped matrix nanocomposites in drug delivery, Chem. Soc. Rev. 43 (1) (2014) 444–472, https://doi.org/10.1039/C3CS60299K.
[90] D. Sun, X. Zhuang, X. Xiang, Y. Liu, S. Zhang, C. Liu, S. Barnes, W. Grizzle, D. Miller, H.G. Zhang, A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes, Mol. Ther. 18 (9) (2010) 1606–1614, https://doi.org/10.1038/mt.2010.105.
[91] M.J. Haney, N.L. Klyachko, Y. Zhao, R. Gupta, E.G. Plotnikova, Z. He, T. Patel, A. Piroyan, M. Sokolsky, A.V. Kabanov, E.V. Batrakova, Exosomes as drug delivery vehicles for Parkinson's disease therapy, J. Control. Release 207 (2015) 18–30, https://doi.org/10.1016/j.jconrel.2015.03.033.
[92] D. Zhang, H. Lee, Z. Zhu, J.K. Minhas, Y. Jin, Enrichment of selective miRNAs in exosomes and delivery of exosomal miRNAs in vitro and in vivo, Am. J. Physiol. Cell. Mol. Physiol. 312 (1) (2017) L110–L121, https://doi.org/10.1152/ajplung.00423.2016.
[93] S.G. Darband, M. Mirza-Aghazadeh-Attari, M. Kaviani, A. Mihanfar, S. Sadighparvar, B. Yousefi, M. Majidinia, Exosomes: natural nanoparticles as bio shuttles for RNAi delivery, J. Control. Release 289 (2018) 158–170, https://doi.org/10.1016/j.jconrel.2018.10.001.
[94] X. Luan, K. Sansanaphongpricha, I. Myers, H. Chen, H. Yuan, D. Sun, Engineering exosomes as refined biological nanoplatforms for drug delivery, Acta Pharmacol. Sin. 38 (6) (2017) 754–763, https://doi.org/10.1038/aps.2017.12.
[95] B.S. Cho, J.O. Kim, D.H. Ha, Y.W. Yi, Exosomes derived from human adipose tissue-derived mesenchymal stem cells alleviate atopic dermatitis, Stem Cell Res Ther 9 (1) (2018) 187, https://doi.org/10.1186/s13287-018-0939-5.
[96] M.N. Oskouie, N.S. Aghili Moghaddam, A.E. Butler, P. Zamani, A. Sahebkar, Therapeutic use of curcumin-encapsulated and curcumin-primed exosomes, J. Cell. Physiol. (2018), https://doi.org/10.1002/jcp.27615 In press.
[97] J. Wang, Y. Yao, X. Chen, J. Wu, T. Gu, X. Tang, Host derived exosomes-pathogens interactions: potential functions of exosomes in pathogen infection, Biomed. Pharmacother. 108 (2018) 1451–1459, https://doi.org/10.1016/j.biopha.2018.09.174.
[98] J. Rezaie, S. Ajezi, Ç.B. Avci, M. Karimipour, M.H. Geranmayeh, A. Nourazarian, E. Sokullu, A. Rezabakhsh, R. Rahbarghazi, Exosomes and their application in biomedical field: difficulties and advantages, Mol. Neurobiol. 55 (4) (2018) 3372–3393, https://doi.org/10.1007/s12035-017-0582-7.