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Minireview

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Cell-Cell Communication *Via* Extracellular Membrane Vesicles and Its Role in the Immune Response

Inkyu Hwang*

The host immune response involves a variety of cell types, including specialized immune and non-immune cells. The delicate coordination among these cells *via* close communication is central for the proper operation of immune system. Cell-cell communication is mediated by a complex network that includes soluble factors such as cytokines, chemokines, and metabolites exported from cells, as well as membrane-bound receptors and their ligands. Cell-cell communication is also mediated by membrane vesicles (e.g., exosomes, ectosomes), which are either shed by distant cells or exchanged by cells that are making direct contact. Intercellular communication *via* extracellular membrane vesicles has drawn much attention recently, as they have been shown to carry various biomolecules that modulate the activities of recipient cells. In this review, I will discuss current views on cell-cell communication *via* extra-cellular membrane vesicles, especially shedded membrane vesicles, and their effects on the control of the immune system.

INTRODUCTION

The host immune system, which has evolved to protect the host against infections with pathogenic agents or toxins, is composed of a variety of types of cells, including both specialized immune cells and non-immune cells. While each type of cell makes a vital and unique contribution to the system, the delicate coordination of these signals is accomplished by close communication between cells. This communication is critical for an optimal immune response: the effective prevention of the spread of infections (and toxins) without eliciting unwanted immunopathologic signs or symptoms (Allison and Eugui, 1983; Cooper, 2009).

Cell-cell communication may take place *via* direct contact. Here, membrane-bound receptors and their cognate ligands may play essential roles (Gomez-Rodriguez et al., 2007; Huang et al., 1999; Hwang and Ki, 2011; Hwang et al., 2000; Peterson, 2003; Vestweber, 2007). The set of receptors and ligands making contact with each other in cells determine the phenotypic changes in those cells during or after the contact. In addition, direct cell-cell contact may allow the directional release of intracellular molecules from a donor cell only to a recipient (target) cell at the interface of the contact (Fooksman et al., 2010). This type of molecule release may minimize unwanted bystander effects of the immune effector molecules on cells in close proximity of the target cell.

Cell-cell communication during immune responses is also mediated by soluble factors exported from cells. Cytokines and chemokines are a group of proteins with immunological functions secreted either by immune or non-immune cells. Like hormones, cytokines spread out from their producing cells to target cells, which express cognate receptors on the surface. The consequences of cognate cytokine-receptor interactions are diverse; many cytokine-receptor interactions are immunostimulatory while others are highly immunosuppressive (Muller, 2006; Xia and Wadham, 2011; Yoshimura and Muto, 2011). The balance between production of immunostimulatory and immunosuppressive cytokines is central for tight immune regulation. Chemokines are a group of chemotactic cytokines that induce recruitment of immune cells to the lymphoid organs or the site of infection and transmigration of those cells across the endothelium of the blood vessels. Precise spatiotemporal expression of both chemokines and chemokine receptors is essential for accurate communication between the cells involved in a specific immune response (Franciszkiewicz et al., 2012; Graham and Locati, 2013).

Cell-cell communication also takes place *via* two primary types of extracellular membrane vesicles (EMVs), termed exosomes and ectosomes (Hwang et al., 2003; Kim et al., 2009; Lee et al., 2012; Ludwig and Giebel, 2012) . Exosomes are nanometric membrane vesicles (40-100 nm in diameter) formed inside cells as a part of multivesicular body (Raposo and Stoorvogel, 2013). In contrast, ectosomes directly bud out of the plasma membrane. A number of studies have documented biological roles of EMVs as mediators of cell-cell communication. Cell-cell communication *via* EMVs has gathered special attention recently among immunologists, as they have been found to carry a variety of immunostimulatory and/or immunosuppressive molecules either on the surface or in the

Research Center for Chemical Biology, KRIBB-RIKEN Global R&D Center Program, Korea Research Institute of Bioscience and Biotechnology, Cheongwon 363-883, Korea
*Correspondence: ihwang@kribb.re.kr

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luminal space (Thery et al., 2009). In this review, I will provide up-to-date information on the biogenesis of EMVs, mechanisms for membrane vesicle exchange, the biological significance of EMVs, and their potential applications.

CELL-CELL COMMUNICATION *VIA* EXOSOMES

Biogenesis and the route of secretion of exosomes

Exosomes are nanometric membrane vesicles naturally formed inside cells as a part of the intracellular endocytic pathway and released to the extracellular space (Huotari and Helenius, 2011). Exosomes are observed as early as the early endosome stage, which occurs immediately after endocytosis (pinocytosis) of extracellular materials at the plasma membrane. As the endocytic process proceeds further, more exosomes are formed inside the larger organelles called late endosomes, or multivesicular bodies (MVBs). Detailed molecular mechanisms for the generation of exosomes are yet to be understood, but it is known that they are formed when the limiting membrane of early and late endosomes invaginates into the luminal space and detaches from the outer membrane (Roxrud et al., 2010). While late endosomes are typically destined to merge with lysosomes for hydrolytic degradation of their cargo by lysosomal enzymes (Fredrickson and Gardner, 2012), many of them bypass that pathway and are guided to the plasma membrane (Huotari and Helenius, 2011). Direct fusion of exosome-containing late endosomes with the plasma membrane allows the exosomes to be released to the extracellular space (Monteiro et al., 2009).

The formation of intraluminal vesicles (ILVs), i.e., exosomes, and the guided migration of endosomes, is accomplished by a complex cellular mechanism comprised of multiple protein complexes and signaling molecules (Boes et al., 2004). The endosomal sorting complex required for transport (ESCRT) is composed of four multi-protein complexes, ESCRT 0, 1, 2 and 3 (Jouvenet, 2012). ESCRT complexes play a central role in the entire process. ESCRT, which interacts with ubiquitinated proteins (Luzio et al., 2009), takes cargo proteins to the late endosomes and assembles them into exosomes. Besides ESCRT, various signaling proteins including Rab small GTP binding proteins and their signaling partners (GEFs and GAPs) (Liu and Storrie, 2012), are deeply involved not only in the formation of exosomes, but also in the release of exosomes to the extracellular space. Late endosomes move along the microtubule network, and kinesin and dynein motor proteins provide the force for transportation (Horgan and McCaffrey, 2011). Proteins belonging to the Rab family (e.g., Rab7) also play roles in concert with their downstream effector proteins (i.e., alix, Tsg101) by determining the directionality of the movement.

Molecular composition of exosomes

The lipid bilayer provides the boundary of the exosome. The orientation of the exosomal membrane is similar to that of the plasma membrane, as determined by the orientation of transmembrane proteins (Denzer et al., 2000; Schorey and Bhatnagar, 2008). The disparity between inside and outside leaflets in the phospholipid composition is, however, less distinct in the exosomal membrane. The flipping of phospholipids from inside to outside (and vice versa) takes place frequently and the asymmetry of the membrane exhibited by the plasma membrane is not restored (Laulagnier et al., 2004). The exosomal membrane is enriched in sphingomyelin, phosphatidylserine, glycolipid GM3 and phosphoethanolamine. The exosomal membrane is also enriched in glycosylphosphatidylinositol (GPI)-anchored proteins such as acethylcholinesterase (AchE), LFA-3/CD58, CD55 and CD59, etc. (Rabesandratana et al., 1998).

A variety of transmembrane proteins are found integrated in the lipid bilayer of exosomes. Exosomes are commonly enriched for a group of proteins (e.g., tetraspanins, transferrin receptors, acetylcholinesterase, etc.) irrespective of their origin. Tetraspanins are a family of proteins with four transmembrane domains (Sala-Valdes et al., 2012). They form a specialized platform in exosomes similar to membrane rafts in the plasma membrane, and are associated with other cell surface receptors, intracellular signaling proteins, and cytoskeletal proteins. They play a role in the recruitment of cargo proteins to the endosomal structures and the exosomes (Pols and Klumperman, 2009). In addition, tetraspanins are responsible for fusion between the exosomal membrane and the plasma membrane of recipient cells (Fanaei et al., 2011). Exosomes also carry proteins associated with endosomal structures, which include LAMP1, LAMP2, and ESCRTs.

Another set of transmembrane proteins that are found in exosomes are expressed in a cell-specific manner. Exosomes derived from T cells express T cell receptors (TCRs), as well as T cell costimulatory and adhesion receptors (Blanchard et al., 2002). In contrast, exosomes derived from B cells express B cell receptors, Fc receptors, MHCII, and others (McLellan, 2009). Exosomes produced from mature dendritic cells express high levels of both MHCI and MHCII, as well as costimulatory and adhesion ligands. In contrast, exosomes from immature DCs show a different protein profile including NFGE8 (a phagocytosis-inducing factor), FasL, and TRAIL (an apoptosis-inducing ligand) (Li et al., 2006). Recent data suggest that exosomes from tumor cells often express immunosuppressive ligands, such as NKG2D, whose expression allows the evasion of tumor cells from T cell and NK cell killing (Clayton et al., 2008).

Exosomes also carry diverse biomolecules in their lumen. These include structural (cytoskeletal) proteins (i.e., actin, tubulin, and proteins associated with them) (Wubbolts et al., 2003), as well as proteins involved in trafficking of early and late endosomes and exosomes (e.g., Rabs, Tsg101, Alix) (Liang et al., 2013). Exosomes are also generally enriched with heat shock proteins and chaperones, represented by Hsp70 and Hsp90 (Chalmin et al., 2010). Exosomes originating from immune cells routinely carry a host of immunoregulatory proteins, such as cytokines (e.g., TNF-α, IL-1, IL-18, etc.) (Clayton et al., 2007; Turola et al., 2012). Of interest, a large portion of proteins in exosomes are found polyubiquitinated (Luzio et al., 2009), suggesting that exosomal proteins are subjected to protein degradation by lysosomal enzymes unless they are released to the extracellular space following fusion of the late endosomes to the plasma membrane

Exosomes as a carrier of RNAs

Besides proteins, exosomes also carry nucleic acids (Chaput and Thery, 2011; Zomer et al., 2010). An earlier study showed that exosomes isolated from the blood of patients chronically infected with hepatitis C virus (HCV) carried HCV-specific RNA species. Later, studies characterizing the RNA species carried by exosomes concluded that only a selected set of mRNAs are incorporated into exosomes, and thus profiles of cellular and exosomal mRNAs are disparate. Moreover, mRNAs carried by exosomes were found to be fully functional and translated into intact proteins in the recipient cells. Exosomes also carry miRNAs. As for mRNAs, only a selected set of miRNAs are present in exosomes, and therefore the profile of exosomal miRNAs is distinct from that of cellular miRNAs. Exosomal miRNAs are

also fully functional; they can modify the levels of target mRNAs and proteins when they successfully penetrate into the cytoplasm of the recipient cells (Gibbings et al., 2009; Rabinowits et al., 2009; Rana et al., 2013).

As described, only chosen sets of cellular mRNAs and miRNAs are incorporated into exosomes. The sorting process is mediated by the same cellular machinery used for the sorting of proteins into late endosomes and exosomes. Argonaute-2 (AGO-2) and GW182 play key roles in the production of miRNAs as components of the RNA-induced silencing complex (RISC) (Kawamata and Tomari, 2010). Recent studies have found that AGO-2 and GW182 are enriched in late endosomes. For example, AGO-2 and GW182 are associated with MVBs that are highly ubiquitinated. The ESCRT complex, a key component of the cellular machinery required to sort cytosolic proteins to the late endosomes, acts by associating with ubiquitinated proteins. Thus, the process of sorting cellular miRNAs into exosomes is hypothesized to occur as ubiquitinated RISC complexes interact with the ESCRT complexes in the late endosome (Gibbings et al., 2009).

Uptake of exosomes

Exosomes that are released to the extracellular space by donor cells may be taken up by the same or different type of cells some distance away, and materials carried on the surface or inside of exosomes are transferred to the recipient cells. While cellular and molecular mechanisms for uptake of exosomes are yet to be comprehended, several mechanisms have been proposed (Clayton et al., 2004; Miyanishi et al., 2007; Nolte-'t Hoen et al., 2009; Rieu et al., 2000; Segura et al., 2007). First, exosomes can be taken up by phagocytosis or pinocytosis. Second, expression of phosphatidylserine (PS), which is found on the outer surface of exosomes, facilitates the binding of exosomes to the surface-expressed T cell immunoglobulin domain and mucine domain protein 1 and 4 (TIM1 and TIM4) found on the surface of activated T cells and macrophages, respectively. Alternatively, lactadherin (also known as milk fat globule epidermal growth factor 8, MFGE8) may form complexes with exosomes via interaction with PS. The exosome-lactadherin complex binds to $\alpha v\beta_3$ or $\alpha v\beta_5$ integrins on the surface of their recipient cells. Third, co-expression of a specific integrin(s) and a protein belonging to the tetraspanin family on the exosome surface allows for two sequential events. The integrin(s) interact with cells expressing cognate ligands, which then allows for tetraspanin-mediated fusion of exosomal membrane with the plasma membrane. For example, the expression of LFA-1 in exosomes allows their interaction with ICAM-1-expressing cells. Tetraspanin-facilitated fusion with the plasma membrane of the recipient cell follows (Fanaei et al., 2011). Besides the mechanisms of exosome uptake described above, other mechanisms must operate, making highly cell type-specific uptake of exosomes possible. Exosome uptake is the least studied area in the field of exosome research and is likely to be the focus of future studies.

Physiological (immunological) roles of exosomes

The physiological roles of exosomes are quite versatile and dependent on the origins of exosomes and the types of recipient cells. Further, physiological condition of the donor cell can alter the effects of exosomes on recipient cells as the composition of molecules carried by exosomes may change.

Exosomes generated by dendritic cells (DCs) have been studied extensively. One earlier study reported that only immature (resting) DCs produced a high level of exosomes; however, later studies have concluded that both mature (activate) and immature DCs produce exosomes at a similar level. Exosomes from mature DCs express the various molecules required for T cell priming (e.g., both MHCI and MHCII, costimulatory ligands, adhesion molecules, etc.) (Bianco et al., 2007; Taieb et al., 2005; Thery et al., 1999). Purified primary resting T cells incubated with exosomes obtained from the culture supernatants of activated DCs, which were loaded with a cognate antigen beforehand, led to full activation/proliferation of T cells (Bianco et al., 2007; Hwang et al., 2003). When T cells are cultured with antigen-loaded exosomes in the presence of DCs, T cell activation/proliferation becomes even more robust. While that result can be interpreted that DCs provide T cells with the bystander costimulatory ligands and cytokines necessary to promote T cell activation, it is also possible that cognate peptides in exosomes are transferred to MHCs intrinsically expressed by DCs after uptake. Indeed, one study has shown that $CD4^+$ T cells are activated only when both exosomes and DCs express MHCII and fail to be activated when DCs lacking MHCII are used (Li et al., 2006).

In contrast to the immunostimulatory exosomes prepared from mature DCs, those derived from immature DCs are rather immunosuppressive (Thery et al., 1999). Compared to exosomes from mature DCs, exosomes from immature DCs express significantly less costimulatory and adhesion ligands. Additionally, they also express immune suppressive ligands such as tumor growth factor-β (TGF-β), NKG2D and galectin-9. Moreover, exosomes derived from immature DCs were found to express death ligands, (e.g., CD95L), which can induce apoptotic cell death of CD95-expressing T cells and attenuate T cell immunity.

B cells also release exosomes expressing both MHCI and II, but their immunogenicity is very poor compared to that of DC-derived exosomes. Exosomes derived from T cells exert multiple functions. Exosomes derived from activated T cells express CD95L to induce killing of bystander T cells, facilitating activation-induced cell death (Blanchard et al., 2002; McLellan, 2009). Notably, exosomes derived from macrophages infected with an intracellular pathogen, such as *Mycobacterium tuberculosis* or *Mycobacterium bovis*, shed exosomes that are transmitted to distant DCs, leading to presentation of pathogen-specific antigens to $CD4^+$ T cells and DC maturation. Similarly, exosomes derived from endothelial cells infected with cytomegalovirus (CMV) convey CMV-specific antigens to DCs to activate $CD4^+$ T cells (Giri and Schorey, 2008; Walker et al., 2009).

Tumor cells secrete exosomes as well. Exosomes from tumor cells can have effects on immune cells, resulting in promotion or suppression of the host immunity to the tumor (Clayton et al., 2007; 2008; Filipazzi et al., 2012; Gastpar et al., 2005; Taylor and Gercel-Taylor, 2011). Earlier studies showed that immunization of mice with exosomes prepared from tumor cells reduced the growth of implanted tumor cells. The immune response elicited by tumor-derived exosomes was found to be antigen-specific. Immunization with exosomes derived from DCs loaded with tumor cell extracts or tumor-specific antigens also induces strong tumor-specific immune responses in mice, hinting that DCs take up tumor-specific antigens and present them on the surface of exported exosomes (Bu et al., 2011).

Several studies have shown the immunosuppressive properties of tumor-derived exosomes as well (Clayton et al., 2008; Raulet et al., 2013). The expression of NKG2D, FasL, and membrane-bound TGF-β, a representative immunosuppressive cytokine, in these exosomes appears responsible for the immunosuppression (Bianco et al., 2007; Borges et al., 2013;

Fagiolo, 2004; Yoshimura and Muto, 2011). TGF-β in tumor-derived exosomes may also induce the development of regulatory T cells and myeloid-derived suppressor cells, which can negatively regulate the overall immune response against tumor cells (Peterson, 2012; Saas and Perruche, 2012).

Research that reveals physiological significances of miRNA transfer *via* exosomes is still in its early stage. Direct evidence that miRNAs transferred to immune cells may change biological properties of the recipient cells are scarce. However, one study has shown the effect of EBV-derived miRNAs in a highly artificial system (Gibbings et al., 2009; Rabinowits et al., 2009; Rana et al., 2013). The impact of miRNA transfer *via* exosomes is therefore one of many future research endeavors.

Generation of exosomes *in vivo*

Studies demonstrating the generation of exosomes *in vivo* are limited. Moreover, results indicating their release *in vivo* are somewhat controversial, as it is difficult to confirm whether those exosome-like structures are naturally made before preparation of tissue samples or an artifact from the preparation of samples. Nevertheless, cell-free vesicles are easily found in body fluids including blood, lymph and synovial fluid etc., and they have the morphology and chemical composition similar to those of exosomes.

Exosomes are found in placenta as well. The level of placenta-derived exosomes increases as the pregnancy reaches full term. The same exosomes are also found in the serum of pregnant women. Both placental and serum exosomes express CD95L and MHCII, and are thought to exert immunosuppressive effects against T cells in CD95L-dependent manner. Those results imply the potential roles of exosomes in immune privileges in the placenta. Tumor-specific exosomes are also found in the blood of tumor patients and their levels increase as the tumor-burden of patients increases (Allan et al., 2006; Almqvist et al., 2008; Bullerdiek and Flor, 2012; Huber et al., 2008; Karlsson et al., 2001).

Some data have shown that DCs release exosomes *in vivo* (Allan et al., 2006). Antigen-sharing between different types of DCs is easily observed in mouse experimental models, and this antigen sharing is thought to take place via exosomes. In addition, antigen transfer *via* exosomes between DCs and non-immune cells, such as intestinal epithelial cells or bronchial cells, are reported as well.

CELL-CELL COMMUNICATION *VIA* ECTOSOMES

Biogenesis and molecular composition of ectosomes

Ectosomes are a class of extracellular membrane vesicles with the size of 50-200 nm in diameter (Sadallah et al., 2011). They are generated by budding directly out of the plasma membrane (Scolding et al., 1989). Polarity of the plasma membrane is maintained during biogenesis of ectosomes. But, different from normal plasma membrane, phosphatidylserine is present at the outer leaflet of the membrane and the symmetry of lipid membrane bilayer is not restored afterward. Molecular rearrangement at the plasma membrane appears to occur during biogenesis of ectosomes; thus, cholesterol and diacylglycerol are highly enriched in ectosomes, and the protein composition of exosomes is distinct from that of the plasma membrane (Campbell and Morgan, 1985).

Ectosome production is generally enhanced when cells are activated by molecules with pathogen-associated molecular patterns (PAMPs,) such as lipopolysaccharide and zymosan A, or through interaction with complement (Combes et al., 2004; Nieuwland et al., 1997). It is of note that ectosomes were first found as a mechanism for release of the complement attack complex from the surface of polymorphonuclear leukocytes (PMNs) (Scolding et al., 1989), which provides the cells with a protection from complement attack. Different from normal cells, many cancer cells constitutively release ectosomes, which may explain their activated phenotypes. Ectosomes may be released *in vivo* from hematopoietic cells and endothelial cells, as they have been found in the blood (Combes et al., 2004; 2005).

PMN-derived ectosomes have been characterized relatively extensively (Gasser and Schifferli, 2005; Gasser et al., 2003; Hess et al., 1999). The formation of ectosomes in PMNs have been observed using electron microscopy. PMN-derived ectosomes express proteins not only from the plasma membrane (e.g., selectins, integrins, complement regulators, HLA-1, etc.) but also from intracellular compartment (e.g., elastase, myeloperoxidase, matrix metalloprotease-9 and proteinase 3). As in other ectosomes, PMN-derived ectosomes also contain PS at the outer leaflet of the membrane and the asymmetry of membrane of those vesicles is not restored.

Biological roles of ectosomes

As with exosomes, the biological roles of ectosomes are highly pleiotropic and dependent on their origins and types of recipient cells (Sadallah et al., 2011). Ectosomes from endothelial cells and monocytes are known to have pro-inflammatory properties, which is mediated in part by IL-1 in the ectosomes. In contrast, ectosomes from cancer cells are rather anti-inflammatory.

While studies on the biological importance of ectosomes are still preliminary, roles of PMN-derived ectosomes have been examined by several groups (Gasser and Schifferli, 2005; Gasser et al., 2003; Hess et al., 2000). PMN-derived ectosomes bind to only a range of selected cells; one study showed that PMN-derived ectosomes only bind to endothelial cells and phagocytic cells. It is also known that PMN-derived ectosomes bind to a soluble factor in plasma, i.e., C1q, whose binding triggers a cascade of complement fixation leading to activation of C3. Given that, it may not be desirable for ectosomes to stay in the blood for a long period of time. Indeed, PMN-derived ectosomes in blood adhere to erythrocytes, and the ectosome-erythrocyte complexes are actively removed in the spleen, liver, and bone marrow in a similar fashion as other immune complexes (Gasser and Schifferli, 2005).

The effects of PMN-derived ectosomes on macrophages have been examined. Incubation of a macrophage cell line with PMN-derived ectosomes attenuates its activation by LPS and zymosan A and reduces the levels of inflammatory cytokines (i.e., IL-8, TNF-β) produced. PS present in the outer surface of ectosomes is found to be responsible for the immunomodulatory property of ectosomes (Gasser and Schifferli, 2004), and a specific receptor protein tyrosine kinase (i.e., Mer receptor tyrosine kinase) is involved in the intracellular signaling process (Scott et al., 2001). PMN-derived ectosomes also have an inhibitory effect on immature DCs (Eken et al., 2008; Gasser and Schifferli, 2004). Thus, when immature DCs are cultured with LPS in the presence of PMN-derived ectosomes, expression levels of the cytokines and costimulatory and adhesion ligands required for T cell activation are significantly reduced, compromising T cell activation.

APPLICATIONS OF EXTRACELLULAR MEMBRANE VESICLES FOR CLINICAL USES

Because exosomes derived from tumor cells were found to

elicit an antigen-specific immune response, those vesicles have been tested as tumor vaccines in preclinical animal studies. Likewise, exosomes obtained from DCs that were loaded with tumor-specific antigens or tumor cell extracts have been used for the same purposes, both with promising successes in animal studies. The mechanisms underlying the potent immune responses mounted by tumor-derived or DC-derived exosomes are still not clear, but it has been suggested that heat shock proteins enriched in those exosomes may have an adjuvant effect on the host immunity by stimulating innate immune cells, such as NK cells. Indeed, both in preclinical and clinical trials using DC-derived exosomes, the activation of NK cells was clearly observed, indicating the importance of NK cells in the use of exosomes for tumor immunotherapy. The use of DC-derived exosomes as a cell-free tumor vaccine has advantages over the use of intact DCs, which include their stability and safety. DC-derived exosomes expressing a tumor-specific antigen are under phase II clinical trial for non-small cell lung cancer (Chaput and Thery, 2011; Viaud et al., 2010; Zech et al., 2012).

DC-derived exosomes have been also tried for treatment of inflammatory diseases, including rheumatoid arthritis. Exosomes derived from immature DCs expressing immunomodulatory cytokines and ligands (i.e., IL-10, IL-4, FasL) have shown a beneficial effect on delayed-type hypersensitivity and inflammation in animal models (Ostman et al., 2005; Tamura et al., 2012).

Exosomes can be used as diagnostic tools for various diseases, as the molecular contents of exosomes in the blood of patients may change depending on the disease status. In addition, they also can be used for the delivery of proteins and RNAs, e.g., miRNAs. Indeed, exosomes carrying specific miRNAs have been used for suppression of tumor growth (Tan et al., 2013).

Both basic and applied research for extracellular membrane vesicles are still in the early stages. Even though there are a few issues and challenges associated with the use of exosomes as a diagnostic or treatment tool, continued studies on basic mechanisms and applications will make the already exciting field even more promising.

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