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REVIEW

An overview of exosomes: From biology to emerging roles in immune response

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ABSTRACT

The discovery that extracellular vesicles are secreted by various cells into extracellular environment has stimulated a large number of studies in order to elucidate their biological activities. Of the released membrane-bound vesicles, specialized nanoparticles called exosomes are currently highlighted. Today, exosomes are known to be associated with diverse pathologies and represent a variety of immune functions. Recent findings that exosomes deliver lipids, cytosolic proteins, mRNA, miRNA and genetic materials to recipient cells, have been a key milestone in the field. During the past few years, many groups have declared that exosomes are naturally present in body fluids as well as their secretion by nearly all cell types. Because of the fact that exosomes have possible functions in wide-range of pathogenesis from immunology to neurobiology, they become interest of biomedicine. Their potential utility in clinical applications as a therapeutic tool or biomarker is an intense area of research to combat numerous diseases. In this review, we summarize influential developments of exosome biology and their biological functions, exclusively on the immune response.

Key words: extracellular vesicles, exosomes, immune response

Introduction

Cells are known to communicate with each other for the purpose of maintaining their functions in multicellular organisms. Intercellular communication can occur by cell-to-cell contact or interchange of secreted specific molecules, such as lipids, proteins or nucleotides [1]. Over the past two decades, the discovery of plasma membrane derived extracellular vesicles (EVs) as a new mechanism for engagement between cells has aided understanding of their significant biological role in eukaryotic cells. EVs are phospholipid bilayer-dependent formations and have been isolated from almost all cell types [2]. They are also naturally present in body fluids, including saliva, blood, breast milk, urine, cerebrospinal fluid, broncho alveolar lavage fluid and semen [3,4]. EVs that convey short peptides, proteins, cytokines, lipids and nucleic acids are various and classified in terms of diverse functions, morphological properties and biogenesis pathways [5]. These extracellular vesicles have been referred to as exosomes, microparticles, apoptotic blebs, prostasomes, oncosomes, tolerosomes and shedding vesicles according to their cellular origin [6].

Classification of Extracellular Vesicles

EVs are lipid bilayer-dependent structures and generated by the endosomal pathway. They are known to be secreted by all human cell types and present in body fluids. EVs are not homogenous populations. EV classification is based on cellular origin, morphology, function and biogenesis [2]. Although nomenclature is still unclear on classification of EVs, four types of EVs have been classified recently according to their biogenesis, which are exosomes, microvesicles, apoptotic bodies and retrovirus-like particles (Table 1) [6,7].

Microvesicles are approximately 100-1000 nm in diameter and their density is still undefined. They are generated by the budding of the plasma membrane and fission into extracellular space. Microvesicles bear diverse nucleic acids and proteins such as actin and tubulin, β1 integrins, VAMP3 (vesicle-associated membrane protein 3) [2]. Size ranges of microvesicles and exosomes generally overlap. However, microvesicles are believed to be larger than exosomes according to recent flow-cytometry studies [8].

Exosomes are approximately 50-100 nm in diameter and formed by exocytosis of multivesicular

Table 1. Classification of extracellular vesicles

Type	Size	Density	Biogenesis	Composition
Microvesicles	100-1000 nm	undefined	Budding of plasma membrane	Actin, tubulin, β1 integrin,VAMP3*, miRNA*
Exosomes	50-100 nm	1.13-1.19 g/mL	Exocytosis of multivesicular bodies	Heat shock proteins, actin, tubulin, MHC molecules*, tetraspannins (CD63, CD81, CD82, CD9), miRNA, mRNA*
Apoptotic bodies	100-5000 nm	1.16-1.28 g/mL	Budding of plasma membrane during apoptosis	Annexin V, C3b*, thrombospondin, any cellular components
Retrovirus-like particles	90-100 nm	1.13-1.16 g/mL	Direct budding of plasma membrane	Retroviral proteins such as Gag*, cytoskeletal proteins, plasma membrane components

*VAMP3, vesicle-associated membrane protein 3; miRNA, microRNA; mRNA, messenger RNA; MHC molecules, major histocompatibility complex; C3b, complement protein 3b; Gag, group-specific antigen.

bodies (MVBs) [2]. Their density is between 1.13 and 1.19 g/mL [9]. Exosomes displaying cup-shaped morphology contain specific molecules including tetraspannins (CD63, CD81, CD82 and CD9), heat shock proteins, MHC (major histocompatibility complex) molecules, actin, tubulin, microRNA (miRNA) and messenger RNA (mRNA) [8,10].

Apoptotic bodies are generated by budding of plasma membrane during apoptosis. Their size range is 100-5000 nm in diameter and density is between 1.16 and 1.28 g/mL. Annexin V, C3b (complement component 3b) and thrombospondin are well-known biomarkers of apoptotic bodies [8,11].

The last group of EVs is retrovirus-like particles (RLPs). These non-infectious vesicles are generated by the direct budding of the plasma membrane [12]. They are 90-100 nm in diameter, with a density between 1.13-1.16 g/mL and they carry retroviral proteins such as Gag (group-specific antigen) as well as cytoskeletal proteins [13,14]. Gag protein can be used as a biomarker to detect RLPs [8].

In this review, we highlight current knowledge about exosomes and their roles in immune response.

Exosomes

Intercellular communication mediated through exosomes was firstly defined by Pan and Johnstone in 1983. They discovered the loss of transferrin receptor during sheep reticulocytes maturation into erythrocytes and observed that the transferrin receptor was encapsulated into nanovesicles of endosomal origin [15]. In the following years, researchers used the term exosome for these nanovesicles having a mean size of 50-100 nm in diameter [2].

Exosomes are currently known to be originated from reticulocytes [16], B cells [17], T cells, dendritic cells, macrophages [18], epithelial cells [19], neuronal cells [20], mast cells [21], fibroblasts [22] and placenta cells [23]. Furthermore, exosomes allied with exosome-like vesicles have been isolated from distinct body fluids such as blood [24], saliva [25], urine [26], semen [27], amniotic fluid [28] and medium of cell line or primary cell cultures [6].

Exosomes were considered to be plasma membrane debris until they were shown to have significant immunological functions in 1996. Raposo *et al.* demonstrated that exosomes are released by B lymphocytes and carry MHC II molecules which mediate to present antigen to CD4+ T cells [17]. Thus, exosomes derived from B cells have been shown to stimulate adaptive immune response. This major breakthrough led to anticipation for implementation of exosomes on immunotherapy although biological functions of exosomes have not been understood completely.

Biogenesis of Exosomes

Exosomes are formed as part of the endolysosomal pathway which consists of endocytic vesicles, early endosomes, late endosomes and lysosomes [10]. The initial step involves endocytosis of proteins on cell surface by inward budding processes, which can

occur in a clathrin-dependent or clathrin-independent manner [29]. The generated endocytic vesicles are transferred to early endosomes having mildly inner acidic pH and located at the outer edge of the cell. Early endosomes convert into late endosomes by altering protein substance and correspondingly reducing inner acidic pH [30]. In order to form MVBs, late endosomes located near the nucleus bud into their endosomal lumen, which leads to intraluminal vesicles (ILVs). Coexistence of MVBs populations having different cholesterol levels within the same cell results in two diverse consequences. While cholesterol-rich MVBs fuse with the plasma membrane to release exosomes, cholesterol-poor MVBs tend to fuse with lysosomes in order to degrade proteins in lysosomal pathway (Figure 1). Formation of exosomes from MVBs is known to occur by two identified pathways so far: 1. Endosomal sorting complex responsible for transport (ESCRT)–dependent mechanism, 2. ESCRT–independent mechanism. ESCRT-dependent mechanism is comprised of four protein complexes, which are ESCRT-0, I, II and III. ESCRT-0 complex both identifies and separates ubiquitinated proteins at the endosomal membrane. ESCRTI and ESCRT-II complexes assist membrane budding and ESCRT-III completes budding process via division of ILVs within MVBs [1]. The studies on oligodendroglial cell line and pigment-producing melanocytes have shown that the ESCRT- independent mechanism is capable of developing MVB formation as an alternative pathway. In oligodendroglial cell lines, exosome formation and releasing that do not depend upon ESCRT complexes are mediated through ceramide. In concordance with this finding, high concentration of ceramide has been shown in exosomes derived from oligodendroglial cells [31]. Although many studies related with biogenesis of exosomes have been performed recently, most of these mechanisms are still unclear.

Composition of Exosomes

The latest achievement on defining exosome via electron microscopy has shown that exosomes share common structure even though they are derived from different cell types. The exosomal lipid bilayer consists of lipid rafts including sphingolipids, ceramide, cholesterol and phosphoglycerides as well as proteins [32,33]. There are over 4400 exosomal proteins identified by mass spectrometry [5]. However, all exosomes are composed of common exosomal

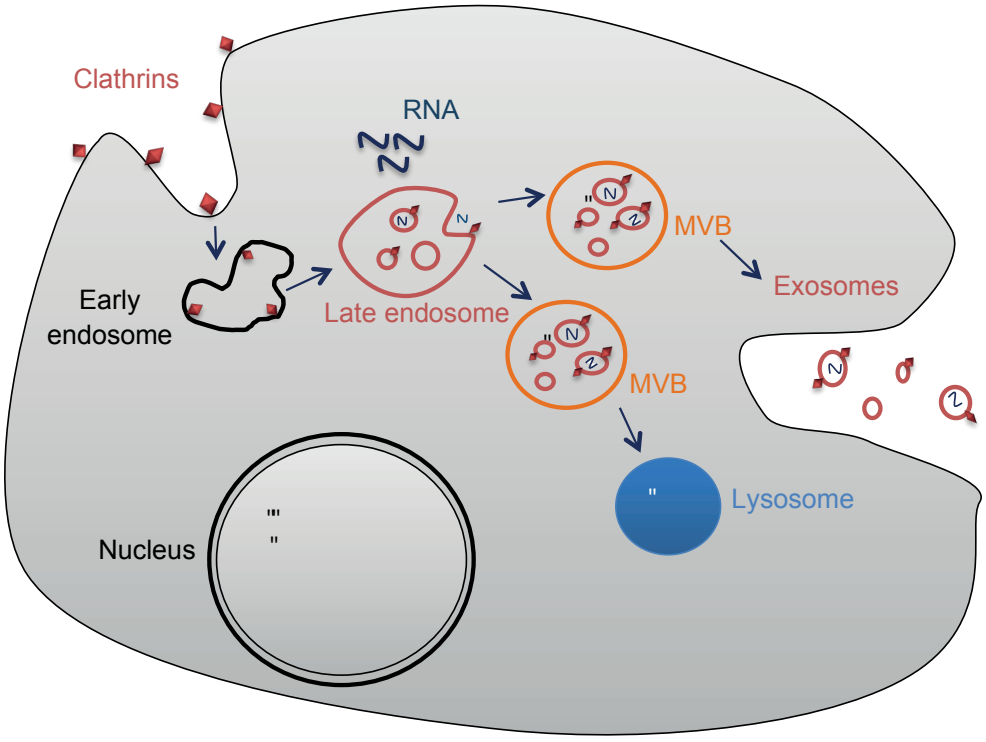

Figure 1. Basic mechanism of exosome biogenesis is illustrated. Here, early endosomes arise through clathrin-mediated endocytosis at the plasma membrane. These vesicles convert into late endosomes by altering protein content and acidification. Afterwards, MVBs (multivesicular bodies) are derived from late endosomes budding reversely into their endosomal lumen and cholesterol-rich MVBs fuse with plasma membrane in order to release exosomes (see text).

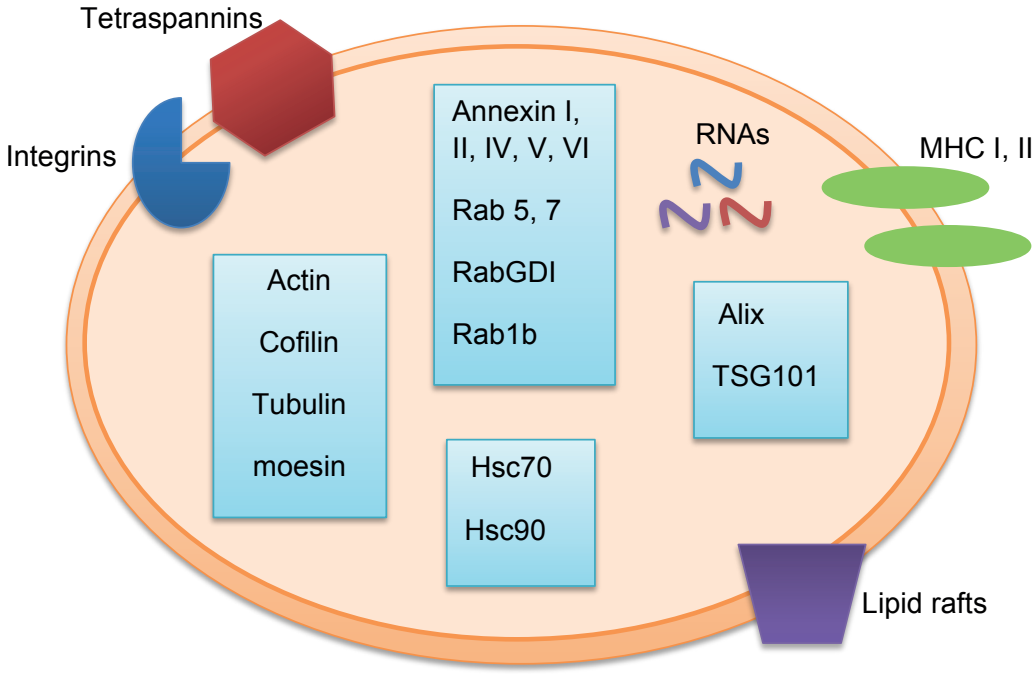

Figure 2. Composition of a DC (dendritic cell)-derived exosome. This schematic representation illustrates an exosome that is generated by lipid rafts, which are originated from cytosol of the producing cell. The proteins that are shown here are related to adhesion, targeting, signal transduction, T-cell stimulation, antigenic peptide binding and membrane fusion (see text). The indicated composition may differ based on parental cell.

membrane-associated proteins such as tetraspannins (CD9, CD63, CD81, CD82, CD151) and integrins, heat shock proteins (Hsc70, Hsc90), ESCRT complex proteins (Alix, TSG101), cytoskeletal proteins (actin, cofilin, tubulin, moesin) and proteins involved in membrane fusion (Annexins I,II,IV,V,VI, Rab5, Rab7, Rabp1B, RabGDI) (Figure 2) [28,34]. Integrins and highly conserved tetraspannins are related with targeting and adhesion. Heat shock proteins are considered to assist protein folding process and antigenic peptide binding. Especially Rab5, Alix and TSG101 are widely used as biomarkers in order to confirm exosome existence [1,5,8]. Further studies revealed that configuration of exosomes depends on cell type of origin and they may perform diverse functions according to origin cell-specific proteins. For instance, due to their MHC class II molecules on membrane, exosomes derived from dendritic cells can interact with T cells [1,35]. Distinctions of tetraspannin complexes are reported to affect target cell selection for exosomes as well [36].

A milestone in the field was the discovery of significant amounts of mRNA, miRNA and small non-coding RNAs in the lumen of exosomes [37]. Transfer of this genetic cargo into target cells by exosomes has currently been an important research area. While some authors indicate that RNA cargo borne by exosomes are different compared to the RNA pool of originated cell, other authors report that they are similar with parental cells [5]. For instance, originating cancer cells and their RNA cargo content are similar. Depending of parental cell and its state, RNA content of exosomes may change [5,38,39]. Proteins, lipids and RNA being identified and generating exosomes are cataloged in the database ExoCarto (http://www.exocarto.org). The list of components is updated with recent novel contributions [40].

Isolation of Exosomes

Body fluids and cell culture mediums are known to contain extracellular vesicles and it is vital to ensure that exosomes are isolated purely from this extracellular vesicle pool. Exosomes can be purified via conventional methods or commercial kits. The most commonly used method of exosome isolation includes a series of centrifugations, often in combination with sucrose gradient ultracentrifugation. Thus, exosomes can be isolated by floatation in sucrose solution according to their density property after performing continuous centrifugations. Ultracentrifugation, when combined with sucrose density gradient, contributes to a high enrichment of exosomes [41]. The obstacle here is the existence

of various nanoparticles and other contaminating material in the similar size range as exosomes in both blood and cell culture media. The probability of overlapping exosomes with equivalent-sized microvesicles, bacteria, virus, mycoplasma, peptides and other small molecules should be considered cautiously [5].

Exosomes can be isolated by ultrafiltration as an alternative way. Ultrafiltration works based on size [42]. Exosomes can also be purified via beads coated with antibody. This antibody targets a known protein on exosomal membrane [43]. Another isolation method is HPLC (High Performance Liquid Chromatography)-based protocol which provides highly pure exosome pellet [5].

Function of Exosomes

Exosomes have been shown to have biological activities *in vivo* and significant roles in various pathological conditions such as cancer [39], autoimmune diseases [2], infectious [44] and neurodegenerative diseases [45]. A number of papers indicate that exosomes are associated with inflammation, coagulation, angiogenesis and apoptosis as well as development and differentiation [5,46].

Johnstone *et al.* reported that exosome secretion is an alternative way to remove plasma membrane proteins during reticulocyte maturation into erythrocyte. Hereby, reticulocytes lacking lysosomes were shown to use exosomes in order to discard unnecessary proteins [16]. Beyond function of releasing waste substances, exosomes act as significant mediators for intercellular communication in between microenvironment of cells or at a distance [47]. They can bind to target-cells through receptor-ligand interactions and transfer their content into cytoplasm by fusing. Exosomes not only bind to recipient cells to transfer proteins or lipids but also deliver nucleic acids such as mRNA, miRNA [1]. Despite the fact that exosomes have various functions physiologically, they are intensively studied as mediators of the immune response.

Exosomes in immune response

The immunological properties of exosomes were firstly reported by Raposo *et al.* This group showed that exosomes derived from Epstein-Barr virus-transformed B cells present MHC class II harbored on their membrane to $CD4^+$ T cells *in vitro* [17]. A few years later, Zitvogel *et al.* noted that dendritic cell (DC)-originated exosomes stimulate $CD8^+$ T cell-dependent anti-tumor immune response in mice *in vivo* [48].

Some papers indicate that exosomes secreted from antigen presenting cells (APCs) directly stimulate T cells in contrast to other reports suggesting that exosomes are required to be captured by APCs for naïve T cell stimulation. Admyre *et al.* revealed that exosomes-harboring MHC class molecules can directly attach to their T cell receptor and trigger $CD4^+$ and $CD8^+$ T cells [49]. On the other hand, Thery *et al.* showed that exosomal MHC-peptide complexes are transferred to DC in order to stimulate naïve T cells [35].

T-cell stimulation via exosomes is known to be based on physiological conditions of origin cell. Mature DC-derived exosomes trigger T-cell activation more efficiently when compared with immature DC-derived exosomes [50,51]. Exosomes are also associated with bearing antigens in order to be degraded. Pathogen-infected cell derived exosomes carry specific antigens from the pathogens and induce $CD4^+$ and $CD8^+$ T cell proliferation [52,53]. Furthermore, it has been demonstrated that tumor cell-derived exosomes have been captured and presented by DCs *in vitro* [54]. When exosomes are secreted by tumor cells releasing cytokines into microenvironment, they activate anti-tumor immune response in mice *in vivo* after injection [55]. On the other hand, some papers suggest that tumor cell-derived exosomes have an opposite effect on immune response by bearing diverse immunosuppressive molecules. This immunosuppressive response is mediated through decreasing $CD4^+$ and $CD8^+$ T cell and natural killer (NK) cell stimulation or enhancing the differentiation of immunosuppressive cells [4]. Injection of exosomes derived from primed tumor cells supported metastasis by decreasing NK cell activation and increasing the differentiation of myeloid cells in mouse models developing melanoma and carcinoma [56]. These controversial *in vivo* results are still unclear if it could be relevant to heterogeneous exosome population.

Various analyses show that exosomes also have anti-inflammatory effects. Kim *et al.* injected exosomes secreted by DC expressing Interleukin-10 (IL-10) to mouse models developing delayed-type hypersensitivity (DTH) and observed that inflammation was suppressed by these DC-derived exosomes [57]. In another study, McDonald *et al.* showed that exosomes derived from macrophages induced with

lipopolysaccharide (LPS) reduced inflammatory pain in mice [58]. The anti-inflammatory functions of exosomes were demonstrated in a drug delivery system as well. Sun *et al.* encapsulated curcumin, an anti-inflammatory drug, into exosomes and injected these exosomes to mouse models suffering with inflammation induced with LPS. Thereafter, the researchers observed that exosomal curcumin provided protection against inflammation [59]. Today, this exosomal drug delivery is still in practice for research applications. However, as a consequence of their biological origin, exosomes are considered to be replaced with liposomes as ultimate goal of immunotherapy approaches.

One of the interesting roles of exosomes in inflammation is that they are capable of stimulating or providing pathological autoimmune responses. Skriner *et al.* noted that citrullinated proteins such as fibrinogen and CD5 antigen-like precursor are carried by synovial exosomes and they are potential autoantigens associated with the pathogenesis of rheumatoid arthritis (RA) [60]. Thus, autoreactive recognition via exosome-associated antigens has been shown in patients with RA. There is other data supporting the evidence that exosomal content may activate autoimmunity such as autoantigens in developing of immune complexes in Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). Kapsogeorgou *et al.* demonstrated that salivary gland exosomes contain Lupus La protein (SS-B), the Smith antigen (Sm) and E3 ubiquitin-protein ligase TRIM21, which are autoantigens associated with SLE and SS [61].

Exosome secretion is regarded as a pathway involved in spreading invading pathogens between cells. Pegtel *et al.* showed that exosomes derived from Epstein-Barr virus (EBV)-infected cells circulate in bloodstream, deliver miRNA cargo and affect healthy recipient cells [44]. Results from Izquierdo-Useros and co-workers indicated that HIV-1 uses DCs as a transit location in the non-replicative stage. The virus packages all viral antigens and particles in exosome-like vesicles after fusing with DCs and acquiring their intracellular vesicle trafficking. Exosomes bearing viral antigens deliver their cargo to $CD4^+$ T cells and provoke infection [62]. Besides viral infections, exosomes are also involved in bacterial infections. Macrophage activation and the release of interferon- γ (INF- γ) are immune response to *Mycobacterium tuberculosis* infection. However, most of infected macrophages are not capable of destroying *M. tuberculosis* because of their INF- γ resistance. 19-kDa lipoprotein and mycolylarabinogalactan peptidoglycan complex, which are mycobacterial proteins, lead to this resistance against the bacteria. The group of Bhatnagar and Beatty declared that exosomes derived from *M. tuberculosis* infected macrophages contain these two mycobacterial substances. Some papers highlight that exosomes may function in two different ways: 1. Initiation of pro-inflammatory molecules by macrophages and stimulation of naïve T cells 2. Enhancing of *M. tuberculosis* survival by damaging macrophage activities [52,63,64,65].

A growing number of studies indicate that exosomes have significant roles in immune tolerance in addition to their roles as immune activators. Exosome-like vesicles derived from prostate gland epithelial cells, known as prostasomes, preserve sperms in seminal fluid by suppressing NK activation in order to prevent immune attack [66,67,68]. Placental explants also release exosomes so that the fetus can be protected from the mother's immune defense system [69]. The presence of exosomes inhibiting T cell activation in milk and colostrum has been reported as well [70]. Exosomes present in bronchoalveolar fluid can create protection against allergens or lead to pro-inflammatory cytokine releasing by airway epithelial cells according to state of host [71]. When tolerosomes released by intestinal epithelium can enhance oral tolerance in rats, they can provoke antigen presentation in some other models [4].

Taken together, exosomes seem to have pleiotropic functions and regulate the immune response depending on the physiological conditions of the organism. The elucidation of these diverse functions in both *in vivo* and *in vitro* environments will lead to improvement of therapeutic and diagnostic applications.

Exosomes as biomarkers

Exosomes are special nanovesicles representing a significant biomedical tool as the future of biomarkers in medicine. All noteworthy findings suggest that exosomes have a crucial role in cell-to-cell communication and degrading unnecessary proteins. Because of their endocytic origin, exosomes carry specialized protein markers such as heat shock proteins (Hsc70 and Hsc90), tetraspannins (CD63, CD9, CD81, and CD82) and Rab family proteins. Exosomal content is a fingerprint of cell type of origin and its

state. Exosomes are also released in diverse body fluids including urine, plasma, breast milk, synovial fluid, bronchoalveolar lavage fluid, amniotic fluid, suggesting that they are information bridges across different body compartments [72]. Some papers recommend that exosome purification may provide much more information than whole body fluid. For instance, urine exosomes isolated from prostate cancer patients contain mRNA of PCA3 (Prostate cancer antigen 3) and TMPRSS2 (Transmembrane protease, serine 2), which are outputs involved in *ERG* (ETS-related gene) fusion chromosomal rearrangement [72,73]. Besides, aquaporin-1 and 2 found on urine exosomes have been described as diagnostic and prognostic markers in the same group of patients. The molecules mentioned above are not detected in whole urine easily [72]. In another research, tumor patients have been shown to have increased level of exosomes expressing CAV1 (Caveolin 1) in their plasma when compared with healthy donors. Furthermore, the discovery of lung tumor-associated miRNAs delivered by plasmatic exosomes and circulation of these miRNAs in bloodstream may represent an early detection of the disease [72,74,75]. The group of Michael and Palanisamy reported that exosomal content from human saliva can be used to detect signs of disease such as SS (Sjögren's syndrome) [76,77] and Keller *et al*. indicate that exosomes secreted by amniotic fluid may also be useful for prenatal diagnosis [28].

All data on exosomes suggest that they are strong key aspects of future medicine as biomarkers. Owing to the fact that exosomes are present in nearly all body fluids and associated with pathogenesis of disease and contain diverse proteins, lipids and nucleic acids, they have a high potential to detect and manipulate diseases.

Conclusion

Exosomes, intensively examined in last few decades, are non-toxic, stable and immunogenic nanovesicles that are associated with biological processes and pathogenesis of some certain diseases. These nanovesicles offering a great potential as therapeutics and biomarkers enable monitoring and diagnosing of various diseases in a non-invasive manner. They have an unique content derived from parental cells and are capable of transferring these molecules between cells without obligation of cell migration. In this emerging field, exosomes may be a powerful clinical tool by manipulating certain level expression of proteins, mRNA or miRNA via bioengineering. The certain distinction between EVs remains a challenge and high-throughput standardized isolation methods are required. However, technological improvement allows detection of these vesicular structures, their functions and their association with diseases in detail. Hopefully, addressing major problems and questions of exosomes will trigger researchers and this promising field.

REFERENCES

[1] **Raposo G, Stoorvogel W.** Extracellular vesicles: Exosomes, microvesicles, and friends. J Cell Biol 2013; 200: 373-383.

[2] **Buzas EI, György B, György N, Falus A, Gay S.** Emerging role of extracellular vesicles in inflammatory diseases. Nat Rev Rheumatol 2014; 10: 356-364.

[3] **Street JM, Barran PE, Mackay CL, Weidt S, Balmforth C, Walsh TS,** *et al*. Identification and proteomic profiling of exosomes in human cerebrospinal fluid. J Transl Med 2012; 10: 5.

[4] **Bobrie A, Colombo M, Raposo G, Théry C.** Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 2011; 12: 1659-1668.

[5] **Vlassov AV, Magdaleno S, Setterquist R, Conrad R.** Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012; 1820: 940-948.

[6] **EL Andaloussi S, Mäger I, Breakefield XO, Wood MJ.** Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 2013; 12: 347-357.

[7] **Van der Pol E, Böing AN, Harrison P, Sturk A, Nieuwland R.** Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 2012; 64: 676-705.

[8] **Akers JC, Gonda D, Kim R, Carter BS, Chen CC.** Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol 2013; 113: 1-11.

[9] **Simons M, Raposo G.** Exosomes: vesicular carriers for intercellular communication. Curr Opin Cell Biol 2009; 21: 575-581.

[10] **Keller S, Sanderson MP, Stoeck A, Altevogt P.** Exosomes: from biogenesis and secretion to biological function. Immunol Lett 2006; 107: 102-108.

[11] **Hristov M, Erl W, Linder S, Weber PC.** Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells in vitro. Blood 2004; 104: 2761-2766.

[12] **Bieda K, Hoffmann A, Boller K.** Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines. J Gen Virol 2001; 82: 591-596.

[13] **Bronson DL, Fraley EE, Fogh J, Kalter SS.** Induction of retrovirus particles in human testicular tumor (Tera-1) cell cultures: an electron microscopic study. J Natl Cancer Inst 1979; 63: 337-339.

[14] **Pincetic A, Leis J.** The Mechanism of Budding of Retroviruses from Cell Membranes. Adv Virol 2009; 2009: 1-9.

[15] **Pan BT, Johnstone RM.** Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell 1983; 33: 967-978.

[16] **Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C.** Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987; 262: 9412-9420.

[17] **Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ,** *et al.* B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996; 183: 1161-1172.

[18] **Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ.** Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. J Cell Sci 2000; 113: 3365-3374.

[19] **Riches A, Campbell E, Borger E, Powis S.** Regulation of exosome release from mammary epithelial and breast cancer cells–a new regulatory pathway. Eur J Cancer 2014; 50: 1025-1034.

[20] **Fauré J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B,** *et al.* Exosomes are released by cultured cortical neurones. Mol Cell Neurosci 2006; 31: 642-648.

[21] **Raposo G, Tenza D, Mecheri S, Peronet R, Bonnerot C, Desaymard C.** Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. Mol Biol Cell 1997; 8: 2631-2645.

[22] **Hu Y, Yan C, Mu L, Huang K, Li X, Tao D,** *et al.* Fibroblast-Derived Exosomes Contribute to Chemoresistance through Priming Cancer Stem Cells in Colorectal Cancer. PLoS One 2015; 10: e0125625.

[23] **Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE,** *et al.* Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J Transl Med 2014; 12: 204.

[24] **Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C.** Exosomal-like vesicles are present in human blood plasma. Int Immunol 2005; 17: 879-887.

[25] **Ogawa Y, Miura Y, Harazono A, Kanai-Azuma M, Akimoto Y, Kawakami H,** *et al.* Proteomic analysis of two types of exosomes in human whole saliva. Biol Pharm Bull 2011; 34: 13-23.

[26] **Pisitkun T, Shen RF, Knepper MA.** Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 2004; 101: 13368-13373.

[27] **Kelly RW, Holland P, Skibinski G, Harrison C, McMillan L, Hargreave T,** *et al.* Extracellular organelles (prostasomes) are immunosuppressive components of human semen. Clin Exp Immunol 1991; 86: 550–556.

[28] **Keller S, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S,** *et al.* CD24 is a marker of exosomes secreted into urine and amniotic fluid. Kidney Int 2007; 72: 1095-1102.

[29] **Van der Goot FG, Gruenberg J.** Intra-endosomal membrane traffic. Trends Cell Biol 2006; 16: 514-521.

[30] **Van Niel G, Porto-Carreiro I, Simoes S, Raposo G.** Exosomes: a common pathway for a specialized function. J Biochem 2006; 140: 13-21.

[31] **Stuffers S, Sem Wegner C, Stenmark H, Brech A.** Multivesicular endosome biogenesis in the absence of ESCRTs. Traffic 2009; 10: 925-937.

[32] **Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF,** *et al.* Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J 2004; 380: 161-171.

[33] **Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Möbius W, Hoernschemeyer J,** *et al.* Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 2003; 278: 10963-10972.

[34] **Hemler ME.** Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol 2005; 6: 801-811.

[35] **Théry C, Duban L, Segura E, Véron P, Lantz O, Amigorena S.** Indirect activation of naïve CD4+ T cells by dendritic cell-derived exosomes. Nat Immunol 2002; 3: 1156-1162.

[36] **Rana S, Yue S, Stadel D, Zöller M.** Toward tailored exosomes: the exosomal tetraspanin web contributes to target cell selection. Int J Biochem Cell Biol 2012; 44: 1574-1584.

[37] **Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO.** Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654-659.

[38] **Zomer A, Vendrig T, Hopmans ES, Van Eijndhoven M, Middeldorp JM, Pegtel DM.** Exosomes: Fit to deliver small RNA. Commun Integr Biol 2010; 3: 447-450.

[39] **Skog J, Wurdinger T, Van Rijn S, Meijer D, Gainche L, Sena-Esteves M,** *et al.* Glioblastoma microvesicles transport RNA and protein that promote tumor growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10: 1470–1476.

[40] **Mathivanan S, Fahner CJ, Reid GE, Simpson RJ.** ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res 2012; 40: D1241-1244.

[41] **Théry C, Amigorena S, Raposo G, Clayton A.** Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol 2006: 3.22.1-3.22.29.

[42] **Cheruvanky A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS,** *et al.* Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. Am J Physiol Renal Physiol 2007; 292: F1657-1661.

[43] **Clayton A, Court J, Navabi H, Adams M, Mason MD, Hobot JA,** *et al.* Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. J Immunol Methods 2001; 247: 163-174.

[44] **Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, Van Eijndhoven MA, Hopmans ES, Lindenberg JL,** *et al.* Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci U S A 2010; 107: 6328-6333.

[45] **Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P,** *et al.* Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proc Natl Acad Sci U S A 2006; 103: 11172-11177.

[46] **Lakkaraju A, Rodriguez-Boulan E.** Itinerant exosomes: emerging roles in cell and tissue polarity. Trends Cell Biol 2008; 18: 199-209.

[47] **Février B, Raposo G.** Exosomes: endosomal-derived vesicles shipping extracellular messages. Curr Opin Cell Biol 2004; 16: 415-421.

[48] **Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D,** *et al.* Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med 1998; 4: 594-600.

[49] **Admyre C, Johansson SM, Paulie S, Gabrielsson S.** Direct exosome stimulation of peripheral human T cells detected by ELISPOT. Eur J Immunol 2006; 36: 1772-1781.

[50] **Segura E, Nicco C, Lombard B, Véron P, Raposo G, Batteux F,** *et al.* ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. Blood 2005; 106: 216-223.

[51] **Montecalvo A, Shufesky WJ, Stolz DB, Sullivan MG, Wang Z, Divito SJ,** *et al.* Exosomes as a short-range mechanism to spread alloantigen between dendritic cells during T cell allorecognition. J Immunol 2008; 180: 3081-3090.

[52] **Bhatnagar S, Schorey JS.** Exosomes released from infected macrophages contain Mycobacterium avium glycopeptidolipids and are proinflammatory. J Biol Chem 2007; 282: 25779-25789.

[53] **Walker JD, Maier CL, Pober JS.** Cytomegalovirus-infected human endothelial cells can stimulate allogeneic CD4+ memory T cells by releasing antigenic exosomes. J Immunol 2009; 182: 1548-1559.

[54] **Wolfers J, Lozier A, Raposo G, Regnault A, Théry C, Masurier C,** *et al.* Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nature Medicine 2001; 7: 297-303.

[55] **Dai S, Zhou X, Wang B, Wang Q, Fu Y, Chen T,** *et al.* Enhanced induction of dendritic cell maturation and HLA-A*0201-restricted CEA-specific CD8(+) CTL response by exosomes derived from IL-18 gene-modified CEA-positive tumor cells. J Mol Med 2006; 84: 1067-1076.

[56] **Liu C, Yu S, Zinn K, Wang J, Zhang L, Jia Y,** *et al.* Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. J Immunol. 2006; 176: 1375-1385.

[57] **Kim SH, Lechman ER, Bianco N, Menon R, Keravala A, Nash J,** *et al.* Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol 2005; 174: 6440-6448.

[58] **McDonald MK, Tian Y, Qureshi RA, Gormley M, Ertel A, Gao R,** *et al.* Functional significance of macrophage-derived exosomes in inflammation and pain. Pain 2014; 155: 1527-1539.

[59] **Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C,** *et al.* A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther 2010; 18: 1606-1614.

[60] **Skriner K, Adolph K, Jungblut PR, Burmester GR.** Association of citrullinated proteins with synovial exosomes. Arthritis Rheum 2006; 54: 3809-3814.

[61] **Kapsogeorgou EK, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN.** Salivary gland epithelial cell exosomes: A source of autoantigenic ribonucleoproteins. Arthritis Rheum 2005; 52: 1517-1521.

[62] **Izquierdo-Useros N, Naranjo-Gómez M, Archer J, Hatch SC, Erkizia I, Blanco J,** *et al.* Capture and transfer of HIV-1 particles by mature dendritic cells converges with the exosome-dissemination pathway. Blood 2009; 113: 2732-2741.

[63] **Beatty WL, Rhoades ER, Ullrich HJ, Chatterjee D, Heuser JE, Russell DG.** Trafficking and release of mycobacterial lipids from infected macrophages. Traffic 2000; 1: 235-247.

[64] **Bhatnagar S, Shinagawa K, Castellino FJ, Schorey JS.** Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response in vitro and in vivo. Blood 2007; 110: 3234-3244.

[65] **9 2007 Nov 1N1 (referaerans) Quah BJ, O'Neill HC.** Mycoplasma contaminants present in exosome preparations induce polyclonal B cell responses. J Leukoc Biol 2007; 82: 1070-1082.

[66] **Ronquist G, Brody I.** The prostasome: its secretion and function in man. Biochim Biophys Acta 1985; 822: 203-218.

[67] **Burden HP, Holmes CH, Persad R, Whittington K.** Prostasomes—their effects on human male reproduction and fertility. Hum Reprod Update 2006; 12: 283-292.

[68] **Tarazona R, Delgado E, Guarnizo MC, Roncero RG, Morgado S, Sánchez-Correa B,** *et al.* Human prostasomes express CD48 and interfere with NK cell function. Immunobiology. 2011; 216: 41-46.

[69] **Hedlund M, Stenqvist AC, Nagaeva O, Kjellberg L, Wulff M, Baranov V,** *et al.* Human placenta expresses and secretes NKG2D ligands via exosomes that down-modulate the cognate receptor expression: evidence for immunosuppressive function. J Immunol 2009; 183: 340-351.

[70] **Admyre C, Johansson SM, Qazi KR, Filén JJ, Lahesmaa R, Norman M,** *et al.* Exosomes with immune modulatory features are present in human breast milk. J Immunol 2007; 179: 1969-1978.

[71] **Prado N, Marazuela EG, Segura E, Fernández-García H, Villalba M, Théry C,** *et al.* Exosomes from bronchoalveolar fluid of tolerized mice prevent allergic reaction. J Immunol 2008; 181: 1519-1525.

[72] **Properzi F, Logozzi M, Fais S.** Exosomes: the future of biomarkers in medicine. Biomark Med 2013; 7: 769-778.

[73] **Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO,** *et al.* Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. Br J Cancer 2009; 100: 1603-1607.

[74] **Logozzi M, De Milito A, Lugini L, Borghi M, Calabrò L, Spada M,** *et al.* High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. PLoS One 2009; 4: e5219.

[75] **Rabinowits G, Gerçel-Taylor C, Day JM, Taylor DD, Kloecker GH.** Exosomal microRNA: a diagnostic marker for lung cancer. Clin Lung Cancer 2009; 10: 42-46.

[76] **Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG,** *et al.* Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis 2010; 16: 34-38.

[77] **Palanisamy V, Sharma S, Deshpande A, Zhou H, Gimzewski J, Wong DT.** Nanostructural and transcriptomic analyses of human saliva derived exosomes. PLoS One 2010; 5: e8577.