Extracellular vesicles: fundamentals and clinical relevance

Wael Nassar[a,c], Mervat El-Ansary[b], Mostafa Abdel Aziz[c], Ehab El-Hakim[d]

[a]Department of Nephrology, Sahel Teaching Hospital, General Organization of Teaching Hospitals and Institutes (GOTHI), [b]Department of Clinical Pathology, Stem Cell Unit, Faculty of Medicine, Cairo University, [c]Department of Nephrology, Kidney Transplantation Unit, October Six University Hospitals, [d]Department of Pediatric Nephrology, Faculty of Medicine, Ain-Shams University, Cairo, Egypt

Correspondence to Wael F Nassar, MD, Hegaz Nephrology Center, 20 Tahreer St; Dokki,11511, Giza, Egypt
Tel: +201222169401
e-mail: hegaz_wn@yahoo.com

All types of cells of eukaryotic organisms produce and release small nanovesicles into their extracellular environment. Early studies have described these vesicles as 'garbage bags' only to remove obsolete cellular molecules. Valadi and colleagues, in 2007, were the first to discover the capability of circulating extracellular vesicles (EVs) to horizontally transfer functioning gene information between cells. These extracellular vesicles express components responsible for angiogenesis promotion, stromal remodeling, chemoresistance, genetic exchange, and signaling pathway activation through growth factor/receptor transfer. EVs represent an important mode of intercellular communication by serving as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, signaling proteins, and RNAs. They contribute to physiology and pathology, and they have a myriad of potential clinical applications in health and disease. Moreover, vesicles can pass the blood–brain barrier and may perhaps even be considered as naturally occurring liposomes. These cell-derived EVs not only represent a central mediator of the disease microenvironment, but their presence in the peripheral circulation may serve as a surrogate for disease biopsies, enabling real-time diagnosis and disease monitoring. In this review, we'll be addressing the characteristics of different types of extracellular EVs, as well as their clinical relevance and potential as diagnostic markers, and also define therapeutic options.

Introduction

Transfer of genetic information between cells had been proposed through only two mechanisms: vertical gene transfer, from parent to the next generation, and horizontal gene transfer, induced through bacteriophages [1] or viruses [2]. Recently, another mechanism of horizontal gene transfer has emerged — namely, naturally occurring cell-derived vesicles such as exosomes and microvesicles. Extracellular vesicles (EVs) are produced constitutively by most, if not all, cell types and, interestingly, contain both, mRNAs and noncoding RNAs such as small regulatory microRNAs (miRNAs) as well as proteins that can be functionally delivered between different cell types and across species [3]. As a result, such vesicles have a significant impact on physiological processes. However, this natural ability of exosomes and microvesicles to transfer genetic information might instead facilitate the spread of disease through the delivery of genetic material and/or pathogenic proteins [4].

EVs are spherical particles enclosed by a phospholipid bilayer. The diameter of vesicles typically ranges from 30 nm to 1 μm [5], the smallest being some 100-fold smaller than the smallest cells. Extracellular vesicles (EVs), or more accurately nanoparticles, is a term used for vesicles that are released from the plasma membrane under basal conditions or during cell stress [6]. The peripheral blood of healthy individuals has been observed to contain ~1010/ml of blood [7,8].

Exosomes are the only vesicles of endocytic origin from inward budding of cell membranes [i.e. multivesicular endosomes (MVEs) [9]]; these vesicles directly fuse with the inner surface of the plasma membrane and release exosomes into the extracellular space [10], whereas microvesicles are shed by direct budding from the plasma membrane to the extracellular space. Confusions exist in the literature on the origin and nomenclature of EVs because vesicles with the size of exosomes that bud at the plasma membrane have also been called exosomes [11]. It should be noted that most studies have not clearly defined the origin of EVs under study; therefore, we will mostly refer to EVs rather than MVs or exosomes. A major ongoing challenge is to establish methods that will allow one to discriminate between exosomes and MVs. Differences in properties such as size, morphology, buoyant density, and protein composition seem insufficient for a clear distinction [12].

The main characteristics of extracellular vesicles
Types of extracellular vesicles

Two common types were distinguished [exosomes and microvesicles (also called shedding vesicles, shedding microvesicles, or nanoparticles)]. A third type, apoptotic vesicles (also called apoptotic blebs or apoptotic bodies), has become a separate class [13]. In addition, 'ectosomes', 'membrane particles', and 'exosome-like

vesicles' were distinguished on the basis of the physical and chemical characteristics of vesicles, including size, density, appearance in microscopy, sedimentation, lipid composition, main protein markers, and subcellular origin [i.e. originating from intracellular compartments (exosomes) or plasma membranes] [14].

The molecular composition of extracellular vesicles

As a consequence of their origin, exosomes from different cell types contain endosome-associated proteins (e.g. Rab GTPase, SNAREs, Annexins, Flotillin, Alix, Tsg101, and Tetraspanins), a family of more than 30 proteins that are composed of four transmembrane domains such as CD63, CD81, CD82, CD53, CD9, and CD37 [15–17]. EVs are also enriched in proteins that associate with lipid rafts, including glycosylphosphatidylinositol-anchored proteins and flotillin [18]. In comparison with the plasma membrane, exosomes from a variety of cells [19] are highly enriched in cholesterol, sphingomyelin, and hexosylceramides at the expense of phosphatidyl–choline and phosphatidyl–ethanolamine. The fatty acids in exosomes are mostly saturated or monounsaturated. However, MVs are generated by direct budding of the cell membrane, with a size ranging from 50 to 1000 nm, and express surface markers such as integrin-β, CD40 ligand, and selectins such as plasma selectins and surface protein receptors that characterize the membrane composition of their cells of origin [20].

A major breakthrough was the demonstration that the cargo of EVs included both mRNA and miRNA and that EVs-associated mRNAs could be translated into proteins by target cells [21]. Recently, analysis of RNA from EVs demonstrated that, in addition to mRNA and miRNA, EVs also contain a large variety of other small noncoding RNA species, including RNA transcripts overlapping with protein coding regions, repeat sequences, structural RNAs, tRNA fragments, vault RNA, Y RNA, piwi RNA (piRNAs), and small interfering RNAs (siRNAs) [22]. Many RNAs that were isolated with EVs were found to be enriched relative to the RNA profiles of the originating cells [23], indicating that RNA molecules are selectively incorporated into EVs [9]. The miRNAs, which are known to regulate more than 80% of all protein-encoding genes and the long noncoding RNA (implicated in the regulation of the epigenome), may induce changes in the cell phenotype. It is therefore conceivable that, under physiologic conditions, EVs may play a critical role in the signaling mechanisms for essential cellular and biological functions [24].

The database ExoCarta (*http://www.exocarta.org*) catalogs proteins, lipids, and RNA that have been identified in EVs from different sources. Currently, ExoCarta lists entries for 13 333 proteins, 2375 mRNAs, 764 miRNA, and 194 lipids associated with EVs [25].

Biogenesis and release of extracellular vesicles

After nascent polypeptides are translated in the ribosomes, polypeptides fold into its characteristic and functional three-dimensional structure in the endoplasmic reticulum [26]. Only properly folded proteins are then transported from the rough endoplasmic reticulum to the Golgi apparatus. Correction of folding of the newly formed proteins is made possible by several endoplasmic reticulum chaperone proteins where glucose, calcium, and redox buffers are required for successful protein folding [3]. It is important here to mention that chaperones are not present when the macromolecules perform their normal biological functions and have correctly completed the processes of folding and/or assembly [27]. Mal-folded proteins are sent back to the cytosol in transient complexes that prevents the protein from further transit and secretion through the endosomal pathway (Fig. 1) [28,29].

The mechanism of formation of exosomes is mediated through the endosomal pathway, including endocytic vesicles, early endosomes, and late endosomes, also known as multivesicular bodies, and lysosomes [30]. The pathway of MVEs that are prone to fuse with lysosomes and predestined for lysosomal degradation differs from the pathway of secretory MVEs predestined to become secreted as exosomes: endosomal sorting complex responsible

Figure 1
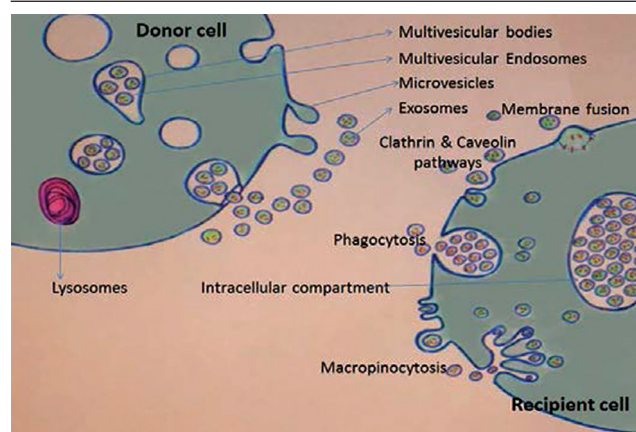
At the 'donor cell': release of microvesicles (MVs) and exosomes. MVs bud directly from the plasma membrane, whereas exosomes are represented by small vesicles of different sizes that are formed as the intraluminal vesicle by budding into early endosomes and multivesicular eendosomes (MVEs) and are released by fusion of MVEs with the plasma membrane. At the 'recipient cell': pathways shown to participate in extracellular vesicle uptake by target cells.

for transport (ESCRT)-dependent and ESCRT-nondependent pathways. The ESCRT comprises four multiprotein complexes assembled within the MVEs: (ESCRT)-0, -I, -II, and -III, with associate accessory proteins (e.g. Alix and VPS4). The ESCRT-0, -I, and -II complexes recognize and sequester ubiquitinated membrane proteins at the endosomal membrane, whereas the ESCRT-III complex is responsible for membrane budding and actual scission of intraluminal vesicles [31–33]. An alternative pathway, independent of the ESCRT machinery, has also been described, and it includes the ceramide and sphingolipid pathway, in which the enzyme sphingomyelinase-2 (nSMase2) is involved in mediation of exosomal release [34,35].

The external membrane of the multivesicular bodies fuses with the plasma membrane of the cell, resulting in release of their segregated vesicles to the extracellular space. The release of large biomolecules through the plasma membrane can occur through the process of exocytosis, which has regulatory and signaling functions. Exocytosis can be either constitutive (non-calcium-dependent) or regulated (calcium-dependent) [36]. Constitutive exocytosis occurs in all cells and serves to secrete extracellular matrix components or incorporate newly synthesized proteins into the plasma membrane following fusion with the transported vesicles.

Disturbances in redox regulation, calcium regulation, glucose deprivation, and viral infection [37] or the overexpression of proteins [38] can lead to endoplasmic reticulum stress, a state in which the folding of proteins slows down. Further increase in unfolded proteins leads to unfolded protein response, which aims at restoring normal function of the cell by halting protein translation, degrading misfolded proteins, and activating the signaling pathways that lead to increasing the production of molecular chaperones involved in protein folding. If these objectives are not achieved within a certain lapse of time or the disruption is prolonged, the unfolded protein response aims toward activation of apoptosis (e.g. p53 pathway). This finding supports the notion that, cell stress, with or without apoptosis, can increase the production of exosomes [39,40].

Mechanisms of action of extracellular vesicles at the target cells

The ability of EVs to interact with recipient cells by fusion of their membranes and/or delivering their contents of proteins, lipids, and RNAs, is still not fully understood [35].

Various mechanisms for EV uptake have been proposed, including clathrin-mediated endocytosis, caveolin-dependent endocytosis, phagocytosis, macropinocytosis, and plasma or endosomal membrane fusion [41]. Regardless of the mechanisms, interaction between EVs and recipient cell membrane may include (i) ligand/receptor binding, (ii) fusion, (iii) internalization of their content, or a combination of these [15]. Cell-type-specific adhesion molecules of EVs can interact with specific cells and deliver their 'cargoes', including bioactive lipids, cytokines, growth factors, receptors, and genetic materials [16,17,42]. EVs may directly activate the recipient cell by acting as signaling complexes [42,43].

When endocytosed, EVs may subsequently fuse with the endosomal delimiting membrane targeted to lysosomes for degradation or release of their functionally active mRNA and miRNA load inside the recipient cell. Messenger RNA (mRNA) can be translated in the recipient cells through rRNA, ensuing in the activation of intracellular pathways, whereas miRNAs, siRNA, and piRNA are known to regulate more than 80% of all protein-encoding genes through gene silencing effect [each miRNA can silence hundreds of protein-encoding genes (mRNA)] [37,44]. Long noncoding RNA incorporated in the epigenome (i.e. implicated in the regulation of the epigenome) may induce changes in the cell phenotype and hence alter the cell products [17,45].

Functions of extracellular vesicles

It has become evident that EVs are important mediators of intercellular communication, being involved in the transmission of biological signals between cells to regulate a diverse range of biological processes. The mechanisms of these effects include transfer/activation of signaling protein receptors or intercellular exchange of proteins and RNAs, which are both recruited to induce phenotypic modulation in the target cells [43,46]. EVs have since been demonstrated to be released by a variety of cells of the immune system, including dendritic cells, macrophages, B cells, T cells, and NK cells. These EVs have been demonstrated to be key mediators/regulators of normal immune responses [47]. For example, one can view tumors as a 'cyber-terrorist' using these EVs to elicit aberrant immune regulation [48]. In addition, pathophysiological roles for EVs are beginning to be recognized in diseases including cancer, infectious diseases, neurodegenerative disorders, etc., highlighting potential novel targets for therapeutic intervention. Moreover, both unmodified and engineered EVs are likely to have applications in macromolecular drug delivery [49].

Extracellular vesicles as immunesuppressants

By transporting ligands and receptors, exosomes can orchestrate cell growth and development, and modulate the immune system. Activated T cells and peripheral blood mononuclear cells release vesicles exposing Fas ligand (FasL), a death receptor ligand, which may have immune regulatory function [50]. During the first trimester of normal pregnancy, trophoblast cells release exosomes exposing FasL, which are capable of inducing Fas-mediated T-cell death, suggesting that exosomes contribute to this immune privilege [51,52]. Immature or suppressive dendriticcells (DCs) reduce adaptive immune activation by inducing T-cell apoptosis and promoting a tolerogenic immune response as seen in murine models of transplantation and autoimmune diseases. Suppressive exosomes may also influence the balance between proinflammatory and anti-inflammatory effector T cells inducing T helper Th17/Th1 cells to differentiate into Th2 and Foxp3+regulatory T cells [53].

Extracellular vesicles as immune stimulants

EVs are used by cells not only to suppress the immune system but also to present antigens, stimulating the immune reaction. Human intestinal epithelial cells release exosomes exposing MHC class I and MHC class II molecules at both the apical and basolateral sides, suggesting a possible involvement of exosomes in the transcellular transport of antigens from the lumen of the gut to immune cells [54,55]. The initiation of T-cell-mediated antitumor immune responses requires uptake, processing, and presentation of tumor antigens by DCs. Exosomes from mouse tumor cells transfer tumor antigens to dendriticcells (DCs) *in vivo* [56]. Exosomes exposing MHC class II antigen complexes are secreted from antigen-loaded DCs when these cells contact antigen-specific CD4 T cells. This secretion is preceded by accumulation of intraluminal vesicles exposing both MHC class II and CD9 in MVEs [57]. Mature dendritic cells also secrete exosomes showing functional peptide-bearing MHC class I and II molecules on their membranes that can directly bind to T-cell receptors and activate CD4+ or CD8+ T cells, inducing an adaptive immune response [58].

Other clinically relevant functions of extracellular vesicles

Role of EVs in inflammation: cell-derived vesicles from stressed cells can trigger the production of proinflammatory cytokines by activating targeted cells independent of the presence or absence of previous infections. This activation results in expression and production of tissue factors and interleukins [59].

The elegant experiment of Deng *et al.* [60] shows that EVs derived from adipose tissue when intravenously injected into wild-type C57BL/6 mice resulted in the development of insulin resistance, whereas the insulin resistance was not marked on injection into TLR-4 knockout B6 mice because of the effect of increased levels of TNF-α and IL-6. On the basis of this experiment, the gradual progressive dissemination of insulin resistance throughout the body may be attributed, at least in part, to EVs released from inflamed adipose tissues, which may open the gate for a new modality of treatment for insulin resistance type 2 diabetic patients [61].

The ability of EVs to modulate the inflammatory response is not limited to blood. Autologous EVs from synovial fluid and EVs from T cells, monocytes, and platelets trigger the production and release of interleukins 6 and 8, matrix metalloproteases, monocyte chemotactic proteins 1 and 2, vascular endothelial growth factor (VEGF), and ICAM-1 by synovial fibroblasts, indicating that these vesicles may enhance the destructive activity of these fibroblasts in rheumatoid arthritis [62].

Tumor growth, metastasis, and angiogenesis and EVs: in a phase I clinical trial, 41 patients with advanced cancer with liver metastasis were treated with intravenous infusion of two doses of bioengineered EVs containing two specific siRNAs against two key proteins involved in the development of cancer cells (VEGF and KSP). The drug (which is bioengineered EVs containing two specific siRNAs against (VEGF and KSP)) was safely administered, and in 11 patients the disease either did not progress or it stabilized after 6 months of treatment, and metastasis to the liver or abdominal lymph glands showed complete regression [63]. Cancer cells can release vesicles containing the Fas-associated death domain, a key adaptor protein that transmits apoptotic signals and becomes lost in many cancer cells [64].

Neurodegenerative disorders: Parkinson's disease is characterized by intracellular aggregates of α-synuclein, the Lewy bodies, in dopaminergic neurons. Recently it was reported that a-synuclein released from cells overexpressing the protein is efficiently transferred to recipient normal cells through exosomes [65]. Moreover, another member of the synuclein family, γ-synuclein, secreted from neuronal cells into exosomes can be transmitted to glial cells, thus promoting the aggregation of intracellular proteins [66]. Alzheimer's disease is known to be characterized by extracellular aggregates of beta amyloid peptides known as amyloid plaques [67], exosome-associated-amyloid peptides that may

be involved in plaque formation and plays a significant role in the pathogenesis and progression of AD [61], and tau proteins, which are mainly secreted through exosomes *in vitro* and *in vivo* [68].

Cardiovascular disorders and EVs: EVs from human atherosclerotic plaques have been shown to mediate the functional transfer of ICAM-1 to endothelial cells of neighboring healthy cells, thereby promoting the adhesion of monocytes and transendothelial migration. Thus, plaque EVs may further facilitate atherosclerotic plaque progression [69].

Kidney disorders and EVs: Autogenic or tissue matched allogeneic Mesenchymal Stem Cells and consequently their EVs were administered to patients who were at high risk of developing severe AKI after open heart surgery in an FDA-approved, Phase I Clinical Trial (*http://www.clinicaltrials.gov*; NCT00733876). Beside its safety, renal function did not deteriorate postoperatively in any of these patients, and no adverse or severe adverse events were observed [70].

Autoimmune disorders and EVs: it has been recently demonstrated that tethering APO2L/TRAIL to the liposome membrane may substantially reduce synovial hyperplasia and inflammation in rabbit knee joints [71]. It has been recently reported that inappropriate clearance of apoptotic vesicles is considered to be the primary cause of developing systemic autoimmune diseases [72].

Conclusion

During the past decade, the interest of physicians and molecular biologists in EVs has expanded logarithmically. EVs are abundantly present in body fluids, carry RNAs, and show regulatory functions that represent a perfect tool for early diagnosis of and therapy against a wide range of diseases. Although the clinical application of EVs remains years away, the significance of their diagnostic and therapeutic potential is not an issue of debate. Deciphering the molecular mechanisms of EV biogenesis and function as well as more accurate and standardized purification methods is required for the implementation of EVs in clinical practice. To help coordinate these enormous challenges, the International Society for Extracellular Vesicles was launched in 2011.

Conflicts of interest
There are no conflicts of interest.

References

1 Valadi H. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9:654–659.

2 Bellingham SA, Hill AF. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases?. Front Physiol 2012; 3:124.

3 Conde-Vancells J, Rodriguez-Suarez E, Embade N, Gil D, Matthiesen R, Valle M, *et al.* Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. J Proteome Res 2008; 7:5157–5166.

4 Morel O, Jesel L, Freyssinet JM, Toti F. Cellular mechanisms underlying the formation of circulating microparticles. Arterioscler Thromb Vasc Biol 2011; 31:15–26.

5 Yuana Y, Oosterkamp TH, Bahatyrova S, Ashcroft B, Garcia Rodriguez P, Bertina RM, Osanto S. Atomic force microscopy: a novel approach to the detection of nanosized blood microparticles. J Thromb Haemost 2010; 8:315–323.

6 Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, *et al.* Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. Nanomedicine 2011; 7:780–788.

7 E van der Pol, Boing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev 2012; 64:676–705.

8 Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009; 9:581–593.

9 Beyer C, Pisetsky DS. The role of microparticles in the pathogenesis of rheumatic diseases. Nat Rev Rheumatol 2010; 6:21–29.

10 Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. J Proteomics 2010; 73:1907–1920.

11 Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 2012; 21:R125–R134.

12 Bobrie A, Colombo M, Raposo G, The´ry C. Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 2011;12:1659–1668.

13 Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigenpresenting vesicles. J Exp Med 1996; 183:1161–1172.

14 Aalberts M, van Dissel-Emiliani FM, van Adrichem NP, van Wijnen M, Wauben MH, Stout TA, Stoorvogel W. Identification of distinct populations of prostasomes that differentially express prostate stem cell antigen, annexin A1, and GLIPR2 in humans. Biol Reprod 2012; 86:82.

15 Rana S, Yue S, Stadel D, Zöller M. Toward tailored exosomes: the exosomal tetraspanin web contributes to target cell selection. Int J Biochem Cell Biol2012; 44:1574–1584.

16 Mulcahy LA, Pink RC, Carter DR. Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles 2014; 3:24641.

17 Corrado C, Raimondo S, Chiesi A, Ciccia F, DeLeo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. Int J Mol Sci 2013; 14:5338–5366.

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

19 Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci USA 2004; 101:13368–13373.

20 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.

21 Asea A, Jean-Pierre C, Kaur P, Rao P, Linhares IM, Skupski D, Witkin SS. Heat shock protein-containing exosomes in mid-trimester amniotic fluids. J Reprod Immunol 2008; 79:12–17.

22 Andre F, Schartz NE, Movassagh M, Flament C, Pautier P, Morice P, et al. Malignant effusions and immunogenic tumour-derived exosomes. Lancet 2002; 360:295–305.

23 Masyuk AI, Huang BQ, Ward CJ, Gradilone SA, Banales JM, Masyuk TV, et al. Biliary exosomes influence cholangiocyte regulatory mechanisms and proliferation through interaction with primary cilia. Am J Physiol Gastrointest Liver Physiol 2010; 299:G990–G999.

24 Kang D, Oh S, Ahn SM, Lee BH, Moon MH. Proteomic analysis of exosomes from human neural stem cells by flow field-flow fractionation and nano-flow liquid chromatography-tandem mass spectrometry.J Proteome Res 2008; 7:3475–3480.

25 DD Taylor, C Gercel-Taylor. The origin, function, and diagnostic potential of RNA within extracellular vesicles present in human biological fluids. Front Genet 2013; 4:142,

26 Kang D, Oh S, Ahn SM, Lee BH, Moon MH. Proteomic analysis of exosomes from human neural stem cells by flow field-flow fractionation and nano flow liquid chromatography-tandem mass spectrometry. J Proteome Res 2008; 7:3475– 3480.

27 Soo CY, Song Y, Zheng Y, Campbell EC, Riches AC, Gunn-Moore F, Powis SJ. Nanoparticle tracking analysis monitors microvesicle and exosome secretion from immune cells. Immunology 2012; 136:192–197.

28 Nolte-'t Hoen EN, van der Vlist EJ, Aalberts M, Mertens HC, Bosch BJ, Bartelink W, et al. Quantitative and qualitative flow cytometric analysis of nanosized cell-derived membrane vesicles. Nanomedicine 2012b; 8:712–720.

29 van der Vlist EJ, EN Nolte-'t Hoen, Stoorvogel W, Arkesteijn GJ, Wauben MH. Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. Nat Protoc2012; 7:1311–1326.

30 Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200:373–383.

31 Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. Leukemia 2006; 20:1487–1495.

32 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.

33 Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F, González MA, et al. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nat Commun 2011; 2:282.

34 Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB, Sullivan ML, Karlsson JM, et al. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. Blood 2012; 119:756–766.

35 Bellingham SA, Coleman BM, Hill AF. Small RNA deep sequencing reveals a distinct miRNA signature released in exosomes from prion-infected neuronal cells. Nucleic Acids Res 2012; 40:10937–10949.

36 Nolte-'t Hoen EN, Buermans HP, Waasdorp M, Stoorvogel W, Wauben MH, PA 't Hoen. Deep sequencing of RNA from immune cell-derived vesicles uncovers the selective incorporation of small non-coding RNA biotypes with potential regulatory functions. Nucleic Acids Res 2012a; 40:9272–9285.

37 Camussi G, PJ. Quesenberry. Perspectives on the potential therapeutic uses of vesicles. Exosomes Microvesicles 2013; 1:6.

38 Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol 2012; 10:e1001450.

39 Valapala M, Vishwanatha JK. Lipid raft endocytosis and exosomal transport facilitate extracellular trafficking of annexin A2. J Biol Chem2011; 286:30911–30925.

40 Kober L, Zehe C, Bode J. Development of a novel ER stress based selection system for the isolation of highly productive clones. Biotechnol Bioeng 2012;109:2599–2611.

41 Gercel-Taylor C, Tullis RH, Atay S, Kesimer M, Taylor DD. Nano particle analysis of circulating cell-derived vesicles in ovarian cancer patients. Anal Biochem 2012; 428:44–53.

42 Ji H, Greening DW, Barnes TW, Lim JW, Tauro BJ, Rai A, et al. Proteome profiling of exosomes derived from human primary and metastatic colorectal cells reveal differential expression of key metastatic factors and signal transduction components. Proteomics 2013; 13:1672–1686.

43 Whiteside TL. Immune modulation of T-cell and NK (natural killer)cell activities by TEXs (tumour derived exosomes). Biochem Soc Trans 2013; 41:245–251.

44 Ivannikov M, et al. Synaptic vesicle exocytosis in hippocampal synaptosomes correlates directly with total mitochondrial volume. J Mol Neurosci 2013; 49:223–230.

45 Parolini I, Federici C, Raggi C, Lugini L, Palleschi S, De Milito A, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem 2009; 284:34211–34222.

46 Atay S, Gercel-Taylor C, Kesimer M, Taylor DD. Morphologic and proteomic characterization of exosomes released by cultured extravillous trophoblast cells. Exp Cell Res 2011a; 317:1192–1202.

47 Frangsmyr L, Baranov V, Nagaeva O, Stendahl U, Kjellberg L, Mincheva-Nilsson L. Cytoplasmic microvesicular form of Fas ligand in human early placenta: switching the tissue immune privilege hypothesis from cellular to vesicular level. Mol Hum Reprod 2005; 11:35–41.

48 Clayton A, Mitchell JP, Court J, Linnane S, Mason MD, Tabi Z. Human tumor-derived exosomes down-modulate NKG2D expression. J Immunol 2008; 180:7249–7258.

49 Andaloussi I Mager S EL, Breakfield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 2013; 12:347–357.

50 Rautou PE, Leroyer AS, Ramkhelawon B, Devue C, Duflaut D, Vion AC, et al. Microparticles from human atherosclerotic plaques promote endothelial ICAM-1-dependent monocyte adhesion and transendothelial migration. Circ Res 2011; 108:335–343.

51 Zhao Y, Jiang Z, Guo C. New hope for type 2 diabetics: Targeting insulin resistance through the immune modulation of stem cells. Autoimmun Rev 2011; 11:137–142.

52 Fafi-Kremer S, et al. Viral entry and escape from antibody-mediated neutralization influence hepatitis C virus reinfection in liver transplantation. J Exp Med 2010; 207:2019–2031.

53 Barsoum RS. Hepatitis C virus: from entry to renal injury — facts and potentials Nephrol Dial Transplant 2007; 22:1840–1848.

54 Feng Z, et al. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature 2013; 496:367–371.

55 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 USA 2010; 107:6328–6333.

56 Meckes DG Jr, Raab–Traub N. Microvesicles and viral infection. J Virol 2011; 85:12844–12854.

57 Corrado C, Raimondo S, Chiesi A, Ciccia F, Leo De G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. Int J Mol Sci 2013; 14, 5338–5366.

58 Demirovic D, Rattan SI. Establishing cellular stress response profiles as biomarkers of homeodynamics, health and hormesis. Exp Gerontol 2013; 48:94–98.

59 Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. J Alzheimers Dis 2001; 3:75–80.

60 Deng ZB, Poliakov A, Hardy RW, Clements R, Liu C, Liu Y, et al. Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance. Diabetes 2009; 58:2498–2505.

61 Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, et al. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early alzheimer disease. J Biol Chem 2012; 287:3842–3849.

62 Azevedo L, Janiszewski M, Pontieri V, Pedro A, Bassi E, Tucci P, Laurindo F. Platelet-derived exosomes from septic shock patients induce myocardial dysfunction. Crit Care 2007; 11:R120.

63 Tabernero J, Shapero GI, Larusso PM, Cervantes A, et al. First in man trial of an siRNA targeting VEGF and KSP in cancer patients with liver involvement. Cancer Discov 2013;14:5338–5366.

64 Zhang H, Liu C, Su K, Yu S, Zhang L, Zhang S, et al. A membrane form of TNF-α presented by exosomes delays T cell activation-induced cell death. J Immunol 2006; 176:7385–7393.

65 Martinez-Lostao L, García-Alvarez F, Basáñez G, Alegre-Aguarón E, Desportes P, Larrad L, et al. Liposome-bound apo2l/trail is an effective treatment in a rabbit model of rheumatoid arthritis. Arthritis Rheum 2010; 62:2272–2282.

66 Liang B, Peng P, Chen S, Li L, Zhang M, Cao D, et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. J Proteomics 2013; 80C:171–182.

67 Takeshita N, Hoshino I, Mori M, Akutsu Y, Hanari N, Yoneyama Y, *et al.* Serum microRNA expression profile: miR-1246 as a novel diagnostic and prognostic biomarker for oesophageal squamous cell carcinoma. Br J Cancer 2013; 108:644–652.

68 Kalra H, Simpson R, Ji H, Aikawa E, Altevogt P, Askenase P, *et al.* Vesiclepedia: A compendium for extracellular vesicles with continuous community annotation. PLoS Biol 2012; 10:e1001450.

69 El-Andaloussi S, Lee Y, Lakhal-Littleton S, Li J, Seow Y, Gardiner C, *et al.* Exosome-mediated delivery of sirna *in vitro* and *in vivo*. Nat Protoc 2012; 7:2112–2126.

70 Anna G, John D, Jean F, LeAnna S, Toegel FE, Reiss GR *et al.* Initial report on a phase I clinical trial: prevention and treatment of post-operative acute kidney injury with allogeneic mesenchymal stem cells in patients who require on-pump cardiac surgery. Cell Ther Transplant 2008/01.

71 Camussi G, Deregibus MC, Bruno S, *et al.* Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 2010; 78:838–848.

72 L Biancone, S Bruno, MC Deregibus, C Tetta, G Camussi. Therapeutic potential of mesenchymal stem cell-derived microvesicles. Nephrol Dial Transplant 2012; 27:3037–3042.