DOI 10.1002/pmic.201200329 *Proteomics* 2013, *13*, 1554–1571

REVIEW

Proteomics, transcriptomics and lipidomics of exosomes and ectosomes

Dong-Sic Choi, Dae-Kyum Kim, Yoon-Keun Kim and Yong Song Gho

Department of Life Science, Pohang University of Science and Technology, Pohang, Republic of Korea

Received: July 31, 2012
Revised: September 13, 2012
Accepted: September 20, 2012

Mammalian cells secrete two types of extracellular vesicles either constitutively or in a regulated manner: exosomes (50–100 nm in diameter) released from the intracellular compartment and ectosomes (also called microvesicles, 100–1000 nm in diameter) shed directly from the plasma membrane. Extracellular vesicles are bilayered proteolipids enriched with proteins, mRNAs, microRNAs, and lipids. In recent years, much data have been collected regarding the specific components of extracellular vesicles from various cell types and body fluids using proteomic, transcriptomic, and lipidomic methods. These studies have revealed that extracellular vesicles harbor specific types of proteins, mRNAs, miRNAs, and lipids rather than random cellular components. These results provide valuable information on the molecular mechanisms involved in vesicular cargo-sorting and biogenesis. Furthermore, studies of these complex extracellular organelles have facilitated conceptual advancements in the field of intercellular communication under physiological and pathological conditions as well as for disease-specific biomarker discovery. This review focuses on the proteomic, transcriptomic, and lipidomic profiles of extracellular vesicles, and will briefly summarize recent advances in the biology, function, and diagnostic potential of vesicle-specific components.

Keywords:
Animal proteomics / Ectosomes / Exosomes / Lipidomics / Transcriptomics

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

As an acellular tool for intercellular communication, mammalian cells secrete nanosized extracellular vesicles either constitutively or in a regulated manner [1–3]. These membrane vesicles are bilayered spherical structures enriched with various bioactive materials including proteins, genetic material (mRNA, microRNA, rRNA, and tRNA), and lipids [4–6]. This extracellular vesicle-mediated communication is evolutionarily conserved from archaea, Gram-negative and -positive bacteria, to eukaryotic cells [7–9].

Various types of mammalian cell, such as platelets, leukocytes, epithelial cells, endothelial cells, and tumor cells, secrete extracellular vesicles [10–13]. Extracellular vesicles are also found in physiological and pathological fluids such as plasma, urine, breast milk, semen, amniotic fluid, saliva, and ascites [14–20]. Extracellular vesicles have been suggested to play multifaceted pathophysiological functions in intercellular communication [1–3, 12, 21]. Mammalian extracellular vesicles are also involved in the delivery of pathogenic loads such as prions and retroviruses [22, 23]. Furthermore, recent studies have drawn attention to the importance of mammalian extracellular vesicles in antitumoral multidrug resistance and their clinical applications as diagnostic tools for and vaccines against cancers [24–26].

The terms "exosomes," "ectosomes," "extracellular membrane vesicles," "shedding vesicles," "membrane particles," "exosome-like vesicles," "exovesicles," "nanoparticles," "microvesicles," "microparticles," "matrix vesicles," "aggrosomes," "oncosomes," "prostasomes," and "tolerosomes" have often been used interchangeably in the literature to

Correspondence: Professor Yong Song Gho, Department of Life Science, Pohang University of Science and Technology, San31 Hyojadong, Pohang, Kyungbuk 790–784, Republic of Korea
E-mail: ysgho@postech.ac.kr
Fax: +82-54-279-8609

Colour Online: See the article online to view Fig. 1 in colour.

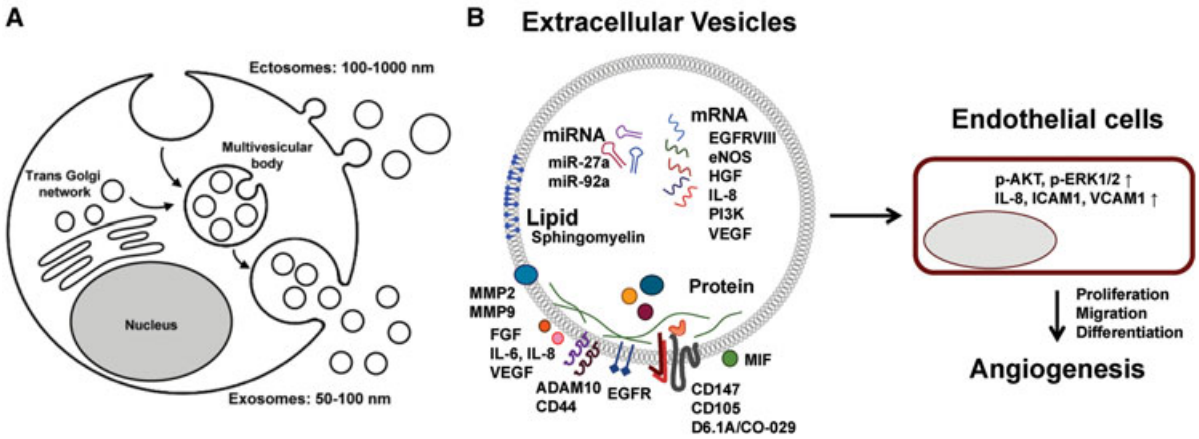

Figure 1. Two types of extracellular vesicle and their roles in angiogenesis. (A) Based on their biogenesis mechanisms, eukaryotic cells secrete two types of extracellular vesicle: exosomes and ectosomes. Exosomes, 50–100 nm in diameter, are secreted by fusion of late endosomes or multivesicular bodies with the plasma membrane. Ectosomes (also known as microvesicles), 100–1000 nm in diameter, are shed directly from the plasma membrane. (B) Endothelial cell activation by extracellular vesicles. These angiogenic activities in vitro and in vivo are mediated primarily by vesicular lipid(s), proteins, mRNAs, and miRNAs.

describe mammalian extracellular vesicles [1]. Based on the mechanisms of biogenesis, mammalian cells secrete two types of extracellular vesicle [1–3]: first, exosomes (50–100 nm in diameter), which are released from the intracellular compartments known as late endosomes or multivesicular bodies after fusion with the plasma membrane (Fig. 1A), and second, ectosomes (also called microvesicles, 100–1000 nm in diameter), which are shed directly from the plasma membrane. Current isolation protocols and analysis parameters of exosomes and ectosomes have been reviewed [27, 28]. Exosomes and ectosomes can be isolated from culture supernatants or body fluids by differential centrifugation, ultrafiltration, ultracentrifugation, or immunoprecipitation [27, 28].

Recent studies have reported that biogenesis of exosome and ectosome is not strictly separate [29–33]. In addition, exosomes and ectosomes can be formed simultaneously, but their ratio differs according to cell type and status (activated or resting) [1–3]. Due to the moderate difference in their physical properties and composition, discriminating between the two types of extracellular vesicles after their release from cells is difficult. Therefore, in this review, we will refer to exosomes and ectosomes collectively as extracellular vesicles. This review focuses primarily on proteomic, transcriptomic, and lipidomic studies of extracellular vesicles that might facilitate understanding of the mechanisms of biogenesis and pathophysiological roles of these complex extracellular organelles.

Table 1. Proteomics, transcriptomics, and lipidomics of extracellular vesicles

		EVpedia	ExoCarta
Proteomes			
Studies	80	61	33
Data sets	112	91[a)]	40
High-throughput proteomes[b)]			
Studies	51	61	15
Data sets	78	91[a)]	19
High-throughput transcriptomes			
mRNA			
Studies	13	12	2
Data sets	17	15	3
miRNA			
Studies	15	4	4
Data sets	27	14	12
Lipidomes			
Studies	12	12	9
Data sets	15	15	10

EVpedia (http://evpedia.info) and ExoCarta (http://exocarta.org) are public online databases of the proteins, mRNAs, miRNAs, and lipids of mammalian extracellular vesicles.
a) EVpedia contains mammalian and nonmammalian eukaryotic and prokaryotic proteomes of extracellular vesicles; ExoCarta contains only mammalian proteomes.
b) High-throughput proteomes in which at least 50 vesicular proteins were identified.

2 Proteomics, transcriptomics, and lipidomics of extracellular vesicles and their implications

Biochemical and biological techniques have been used to identify the protein, mRNA, miRNA, and lipid contents of vesicles [27, 28]. To date, numerous high-throughput proteomic and transcriptomic studies of extracellular vesicles derived from various cell types and body fluids have catalogued several thousand vesicular proteins [10–12, 15–17, 20–22, 34–75] and RNAs [75–100], while few investigators have performed lipidomic profiling of extracellular vesicles [33, 40, 101–110] (Table 1). Although extracellular vesicles

contain a common set of components, one of most interesting aspects is the different findings obtained from different studies and data sets. This may be due to differences in vesicle purification techniques, as well as in the biological fluids/cell types studied and the state of the cell/host that produces extracellular vesicles. For example, when LIM1863-derived extracellular vesicles were isolated using a variety of methods (ultracentrifugation, OptiPrep density gradient ultracentrifugation, or an anti-EpCAM affinity column), significant differences in their proteomes were identified [44]. Furthermore, extracellular vesicles derived from metastatic colorectal cancer cells (SW620) are enriched with cancer progression associated proteins (dipeptidase 1, metastasis associated in colon cancer protein 1, and prominin-1) compared to those of the nonmetastatic colorectal cancer cell line SW480 [74]. Several reviews concerning this subject have been recently published [4–6].

Extracellular vesicles harbor a specific subset of proteins, mRNAs, miRNAs, and lipids rather than random cellular components. Moreover, extracellular vesicles contain both a common set of components and cell type specific protein, mRNA, miRNA, and lipid subsets [1–6, 27]. Identification of common vesicular components will increase our understanding of the molecular mechanisms involved in vesicle cargo-sorting and biogenesis. In addition, the vesicle- and cell type specific cargos of extracellular vesicles are likely linked to their biological and pathophysiological functions (reviewed by Raimondo et al. [27]) and may prove to be a rich source of biomarkers [27, 111, 112]. For example, cancer cell- and endothelial cell-derived extracellular vesicles stimulate endothelial cells by receptor activation and transfer of vesicular cargos, resulting in the progression of angiogenesis in vitro and in vivo [12, 76–79, 113–119]. These angiogenic activities are mediated by vesicular lipids, proteins, mRNAs, and miRNAs (Fig. 1B).

Currently, two public online databases of comprehensive vesicle-specific components (EVpedia and ExoCarta) are available (Table 1). Both EVpedia (http://evpedia.info) and ExoCarta (http://exocarta.org) provide information regarding the mRNAs, miRNAs, and lipids of mammalian extracellular vesicles [120]. ExoCarta contains only the proteomes of mammalian extracellular vesicles, while EVpedia collects nonmammalian eukaryote and prokaryote, as well as mammalian data sets. EVpedia also provides an array of tools, such as Gene Ontology (GO) enrichment analyses and network analyses of vesicular proteins and a comparison of vesicular protein data sets by ortholog identification. These online databases facilitate the sharing and cataloging of data regarding vesicle-specific proteins, nucleic acids, and lipids derived from various types of cells and body fluids. The list of proteins, mRNAs, miRNAs, and lipids found in extracellular vesicles within both EVpedia and ExoCarta is continuously expanding. Authors are invited to report their findings so as to enlarge these databases and thus enhance their utility.

3 Proteomic analysis of extracellular vesicles

During the last 20 years, vesicular proteins have been extensively investigated by MS-based proteomic analysis, Western blotting, fluorescence-activated cell sorting, and immunoelectron microscopy [12, 102, 121–123]. MS-based proteomic tools have played a particularly important role in improving our understanding of the protein compositions of extracellular vesicles from various cell types and body fluids (Table 1). The vesicular proteomes provide diverse information regarding the biogenesis mechanisms and pathophysiological functions of extracellular vesicles and facilitate biomarker discovery based on the protein signature of the originating cells. Recent reviews of this subject are available [27, 111].

In early MS-based proteomic studies of extracellular vesicles [124–127], vesicular proteins were separated using gel electrophoresis, and then highly abundant proteins (14–3-3 proteins, actin, annexins, HSPs, and tetraspanins) were identified by MS. However, in recent high-throughput proteomic studies, increasing numbers of vesicular proteins have been identified by LC-ESI-MS/MS analyses combined with protein and/or peptide separation technologies (Table 2). More recently, extracellular vesicles purified by density gradient ultracentrifugation [27, 28], immunoaffinity columns [35, 44, 45], gel filtration [38], or flow field-flow fractionation [43] have been used in high-throughput proteomic studies (Table 2).

As more vesicular proteomes are identified, it has become apparent that the inclusion or exclusion of cellular proteins into extracellular vesicles is not based on the abundance of the protein, but rather on a controlled protein-sorting mechanism during production of extracellular vesicles. Annotation of vesicular proteomes according to their subcellular distribution revealed that plasma membrane and cytoplasmic proteins are more commonly sorted into extracellular vesicles compared to those of the nucleus and mitochondrion [27, 128]. Several mechanisms of protein sorting into extracellular vesicles during their biogenesis have been proposed [129]: cargo proteins, for example, ubiquitinylated membrane proteins sorted by endosomal-sorting complexes required for transport; co-sorting by protein interaction; co-sorting by lipid interaction; and nonspecific engulfment of cytosolic proteins. However, the molecular mechanisms by which proteins are loaded into extracellular vesicles are not fully understood. Recently, we validated experimentally the co-sorting of cytoplasmic proteins with vesicular cargo proteins via protein–protein interactions, suggesting that direct interactions between cellular proteins are critical for cargo protein sorting during vesicle formation [21].

Proteomic studies on extracellular vesicles of various origins suggest that extracellular vesicles contain both a common set of components and cell type specific protein, mRNA, miRNA, and lipid subsets (reviewed by Raimondo et al. [27]). Common vesicular proteins are known to be involved in

Table 2. High-throughput proteomic studies on extracellular vesicles

	Species	Isolation strategies	Proteomic analysis strategies	Published year	Reference
Cell types					
Hematopoietic cells					
Microglia cell (N9)	*Mus musculus*	Filtration (0.22 μm) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2005	[36]
Platelet	*Homo sapiens*	Preclearing (710 × *g*) > Pelleting (150 000 × *g*)	1DE-LC-ESI-MS/MS	2005	[10]
T-lymphocytic cell, B cell	*Homo sapiens*	Preclearing (750 × *g*, 1500 × *g*) > Pelleting (15 000 × *g*)	1DE-LC-ESI-MS/MS	2006	[11]
Mast cell (MC9)	*Mus musculus*	Preclearing (500 × *g*, 16 500 × *g*) > Filtration (0.22 μm) > Pelleting (120 000 × *g*)	1DE-LC-ESI-MS/MS	2007	[75]
Red blood cell	*Homo sapiens*	Microvesicle: Preclearing (1550 × *g*) > Pelleting (40 000 × *g*) Nanovesicle: Preclearing (1550 × *g*, 40 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2008	[39]
B cell	*Homo sapiens*	Preclearing (750 × *g*, 1000 × *g*) > Pelleting (15 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[34]
Monocyte (THP-1)	*Homo sapiens*	Preclearing (750 × *g*, 1500 × *g*) > Pelleting (16 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[37]
Platelet	*Homo sapiens*	Preclearing (5000 × *g*) > Pelleting (130 000 × *g*) > Gel filtration	2DLC-ESI-MS/MS	2009	[38]
B cell (RN HLA-DR15$^+$)	*Homo sapiens*	Preclearing (200 × *g*, 500 × *g*, 10 000 × *g*) > Pelleting (70 000 × *g*) > Sucrose density ultracentrifugation (190 000 × *g*) > Affinity purification (anti-MHC II)	1DE-LC-ESI-MS/MS	2010	[35]
Reticulocyte	*Rattus norvegicus*	Preclearing (20 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2011	[40]
Thymocyte	*Mus musculus*	Preclearing (2000 × *g*) > Filtration (0.8 μm) > Pelleting (12 200 × *g*)	LC-ESI-MS/MS	2011	[41]
Stem cells					
Neural stem cell (HB1.F3)	*Homo sapiens*	Preclearing (1000 × *g*) > Concentration (100 kDa) > Flow field-flow fractionation	LC-ESI-MS/MS	2008	[43]
Mesenchymal stem cell	*Homo sapiens*	Preclearing (500 × *g*, 800 × *g*) > Concentration (100 kDa) > Sucrose cushion ultracentrifugation (100 000 × *g*) > OptiPrep density gradient ultracentrifugation (150 000 × *g*)	1DE-LC-ESI-MS/MS	2012	[42]
Tumor cells					
Colorectal cancer cell(HT29)	*Homo sapiens*	Preclearing (500 × *g*, 800 × *g*) > Concentration (100 kDa) > Sucrose cushion ultracentrifugation (100 000 × *g*) > Sucrose density gradient ultracentrifugation (150 000 × *g*)	1DE-LC-ESI-MS/MS	2007	[12]
Colorectal cancer cell (LIM1215)	*Homo sapiens*	Preclearing (400 × *g*, 1900 × *g*) > Filtration (0.1 μm) > Concentration (5 kDa) > Pelleting (100 000 × *g*), or (ii) Preclearing (400 × *g*, 1900 × *g*) > Concentration (5 kDa) > Affinity purification (anti-A33)	1DE-LC-ESI-MS/MS	2009	[45]
Colorectal cancer cell (LIM1863)	*Homo sapiens*	Preclearing (480 × *g*, 2000 × *g*) > Filtration (0.1 μm) > Concentration (5 kDa) > Pelleting (100 000 × *g*), (ii) Preclearing (480 × *g*, 2000 × *g*) > Filtration (0.1 μm) > Concentration (5 kDa) > OptiPrep density gradient ultracentrifugation (100 000 × *g*) > Pelleting (100 000 × *g*), or (iii) Preclearing (480 × *g*, 2000 × *g*) > Filtration (0.1 μm) > Concentration (5 kDa) > Affinity purification (anti-EpCAM)	1DE-LC-ESI-MS/MS	2012	[44]
Colorectal cancer cell(SW480, SW620)	*Homo sapiens*	Preclearing (500 × *g*, 2000 × *g*) > Concentration (100 kDa) > Sucrose cushion ultracentrifugation (100 000 × *g*) > OptiPrep density gradient ultracentrifugation (200 000 × *g*)	IEF-LC-ESI-MS/MS	2012	[74]

Table 2. Continued

	Species	Isolation strategies	Proteomic analysis strategies	Published year	Reference
Colorectal cancer cell(HT29)	*Homo sapiens*	Preclearing (500 × *g*, 800 × *g*) > Concentration (100 kDa) > Sucrose cushion ultracentrifugation (100 000 × *g*) > Sucrose density gradient ultracentrifugation (150 000 × *g*)	1DE-LC-ESI-MS/MS	2012	[21]
Other cells					
Neuroglial cell (Mov)	*Mus musculus*	Preclearing (3000 × *g*, 4500 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose cushion ultracentrifugation (100 000 × *g*)	1DE-LC-ESI-MS/MS	2004	[22]
Adipocyte (3T3-L1)	*Homo sapiens*	Preclearing (1000 × *g*, 15 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose density gradient ultracentrifugation (200 000 × *g*)	LC-ESI-MS/MS	2007	[46]
Oligodendrocyte	*Mus musculus*	Preclearing (60 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose density gradient ultracentrifugation (100 000 × *g*) > Pelleting (100, 000 × *g*)	LC-ESI-MS/MS	2007	[53]
Osteoblast (MC3T3-E1)	*Homo sapiens*	Preclearing (2000 × *g*, 20 000 × *g*) > Pelleting (100 000 × *g*)	2DLC-ESI-MS/MS	2007	[54]
Cryptococcus neoformans	*Cryptococcus neoformans*	Preclearing (4000 × *g*, 15 000 × *g*) > Concentration (100 kDa) > Preclearing (4000 × *g*, 15 000 × *g*) > Filtration (0.8 μm) > Pelleting (100 000 × *g*)	LC-ESI-MS/MS	2008	[47]
Endothelial cell (HUVEC)	*Homo sapiens*	Preclearing (200 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2008	[49]
Hepatocyte	*Rattus norvegicus*	Preclearing (500 × *g*) > Filtration (0.22 μm) > Preclearing (10 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2008	[51]
Endothelial progenitor cell	*Homo sapiens*	Preclearing (400 × *g*, 12 500 × *g*) > Pelleting (20 500 × *g*)	1DE-LC-ESI-MS/MS	2009	[50]
Keratinocyte	*Homo sapiens*	Preclearing (6000 × *g*) > Concentration (100 kDa) > Sucrose/D2O cushion ultracentrifugation (100 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[52]
Pancreatic beta cell (NIT-1)	*Mus musculus*	Preclearing (100 × *g*) > Pelleting (22 300 × *g*) > Sucrose density gradient ultracentrifugation (200 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[55]
Saccharomyces cerevisiae	*Saccharomyces cerevisiae*	Preclearing (4000 × *g*, 15 000 × *g*) > Concentration (100 kDa) > Preclearing (4000 × *g*, 15 000 × *g*) > Filtration (0.8 μm) > Pelleting (100 000 × *g*)	LC-ESI-MS/MS	2010	[56]
Drosophila melanogaster (KC167, S2)	*Drosophila melanogaster*	Preclearing (5000 × *g*, 10 000 × *g*) > Sucrose cushion ultracentrifugation (100 000 × *g*) > Sucrose density gradient ultracentrifugation (100 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2011	[48]
Tabecular meshwork cell	*Homo sapiens*	Preclearing (10 000 × *g*) > Pelleting (100 000 × *g*)	2DLC-ESI-MS/MS	2011	[57]
Trophoblast cells (Sw71)	*Homo sapiens*	Preclearing (400 × *g*, 15 000 × *g*) > Concentration (500 kDa) > Pelleting (100 000 × *g*) > Sucrose density gradient ultracentrifugation (222 228 × *g*) > Pelleting (105 000 × *g*)	LC-ESI-MS/MS	2011	[58]
Body fluids					
Seminal fluid	*Homo sapiens*	Preclearing (1000 × *g*) > Pelleting (105 000 × *g*)	LC-ESI-MS/MS	2003	[17]
Urine	*Homo sapiens*	Preclearing (17 000 × *g*) > Pelleting (200 000 × *g*)	1DE-LC-ESI-MS/MS	2004	[15]
Breast milk	*Homo sapiens*	Preclearing (300 × *g*, 3000 × *g*) > Filtration (1.2, 0.8, 0.4, 0.2 μm) > Preclearing (10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose density gradient ultracentrifugation (79 000 × *g*)	LC-ESI-MS/MS	2007	[16]

Table 2. Continued

	Species	Isolation strategies	Proteomic analysis strategies	Published year	Reference
Urine	*Homo sapiens*	Preclearing (250 × *g*, 17 000 × *g*) > Pelleting (200 000 × *g*)	1DE-LC-ESI-MS/MS	2008	[68]
Carotid atherosclerotic plaque	*Homo sapiens*	Preclearing (400 × *g*, 12 500 × *g*) > Pelleting (20 500 × *g*)	1DE-LC-ESI-MS/MS	2009	[59]
Ductal parotid saliva	*Homo sapiens*	Preclearing (300 × *g*, 10 000 × *g*) > Pelleting (200 000 × *g*)	2DLC-ESI-MS/MS	2009	[60]
FBS	*Bos taurus*	Preclearing (20 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[61]
Plasma	*Homo sapiens*	Filtration (0.22 μm) > Gel exclusion chromatography > Sucrose gradient ultracentrifugation (175 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose cushion ultracentrifugation (175 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[63]
Seminal fluid	*Homo sapiens*	Preclearing (1000 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose density gradient ultracentrifugation (78 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[67]
Urine	*Homo sapiens*	Preclearing (17 000 × *g*) > Pelleting (200 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[69]
Urine	*Homo sapiens*	Preclearing (15 000 × *g*) > Filtration (8 μm) > Pelleting (150 000 × *g*) > Sucrose/D2O density ultracentrifugation (200 000 × *g*)	1DE-LC-ESI-MS/MS	2009	[70]
Plasma	*Homo sapiens*	Preclearing (1500 × *g*, 15 000 × *g*) > Pelleting (200 000 × *g*)	SCX-MALDI-MS/MS	2010	[64]
Urine	*Rattus norvegicus*	Preclearing (1500 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose/D2O cushion ultracentrifugation (100 000 × *g*) > Pelleting (100 000 × *g*)	1DE-LC-ESI-MS/MS	2010	[71]
Ascites	*Homo sapiens*	Preclearing (3200 × *g*) > Sucrose cushion ultracentrifugation (100 000 × *g*) > OptiPrep density gradient ultracentrifugation (200 000 × *g*) > Sucrose cushion ultracentrifugation (100 000 × *g*) > OptiPrep density gradient ultracentrifugation (200 000 × *g*)	1DE-LC-ESI-MS/MS	2011	[20]
Plasma	*Rattus norvegicus*	Preclearing (1500 × *g*, 5000 × *g*) > Concentration (0.45 μm) > Pelleting (200 000 × *g*)	1DE-LC-ESI-MS/MS	2011	[65]
Urine	*Homo sapiens*	Preclearing (17 000 × *g*) > Pelleting (200 000 × *g*) > Sucrose density gradient ultracentrifugation (270 000 × *g*) > Pelleting (200 000 × *g*)	1DE-LC-ESI-MS/MS	2011	[72]
Milk	*Bos taurus*	Preclearing (10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose cushion ultracentrifugation (200 000 × *g*) > Sucrose density gradient ultracentrifugation (112 700 × *g*) > Pelleting (200 000 × *g*)	2DLC-ESI-MS/MS	2012	[62]
Saliva	*Homo sapiens*	Preclearing (2600 × *g*) > Filtration (5 μm) > Pelleting (20 000 × *g*)	1DE-LC-ESI-MS/MS	2012	[66]
Urine	*Homo sapiens*	Preclearing (17 000 × *g*) > Pelleting (200 000 × *g*)	1DE-LC-ESI-MS/MS and 2DLC-ESI-MS/MS	2012	[73]

1DE, one-dimensional electrophoresis; 2DLC, two-dimensional LC; SCX, strong cation exchange.

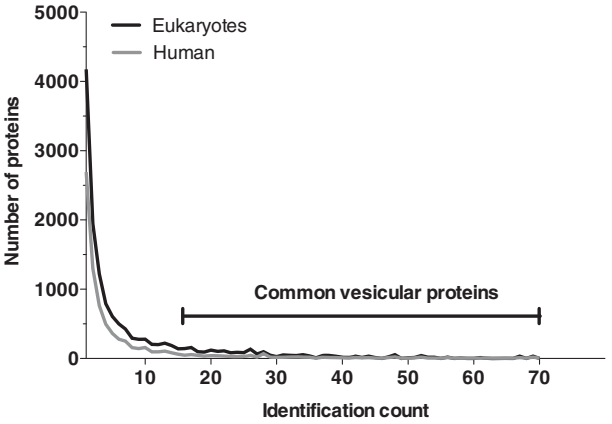

Figure 2. The distribution of identification counts in vesicular proteomes. By combining the 78 qualified high-throughput proteomic data sets (Supporting Information Table S1), we constructed a comprehensive eukaryotic extracellular vesicle proteome data set (Supporting Information Table S2). The distribution of identification counts in 13 718 eukaryotic vesicular proteins, including 7806 human proteins is shown. The identification count of each vesicular protein represents the number of identifications of that protein or its orthologs in 78 high-throughput proteomic data sets. Approximately 2000 eukaryotic vesicular proteins and 797 human vesicular proteins are categorized as common vesicular proteins that are present in at least 16 proteomic data sets.

vesicle structure, biogenesis, and trafficking, while cell type specific vesicular proteins are involved in cell type associated pathophysiological functions.

To provide a global outlook on the molecular functions and interrelationships of vesicular proteins, we constructed a comprehensive proteome data set of eukaryotic extracellular vesicles. We selected 78 qualified high-throughput proteomic data sets that identified at least 50 vesicular proteins (Table 2 and Supporting Information Table S1). From this merged data set, we identified ~13 000 eukaryotic vesicular proteins, including 7806 human proteins (Supporting Information Table S2). We then calculated the identification count of each vesicular protein, which represents the number of identifications of that protein or its orthologs in the 78 proteomic data sets (Supporting Information Table S2). The distribution of the identification counts of vesicular proteins is shown in Fig. 2. We finally categorized ~2000 eukaryotic vesicular proteins, including 797 common human vesicular proteins, as shared by more than 16 proteomic data sets (Supporting Information Table S2). The tetraspanins (CD9, CD63, and CD81), integrins, HSPs, annexins, Tsg101, Alix, Syntenin-1, Rab proteins, cytoskeletal proteins (actins, cofilin-1, ezrin/radixin/moesin, profilin-1, and tubulins), metabolic enzymes (enolases, glyceraldehyde 3-phosphate dehydrogenase, peroxiredoxins, and pyruvate kinase), and ribosomal proteins were categorized as common vesicular proteins, as has been reported elsewhere (reviewed by Raimondo et al. [27]).

When compared with the 7806 total human vesicular proteins, the 797 common proteins were enriched with those originating from the cytoskeleton, cytosol, plasma membrane, and vesicles (Fig. 3A). Furthermore, annotation of these common vesicular proteins according to biological process revealed significant associations with cell motion, cytoskeleton organization, protein localization, regulation of apoptosis, small GTPase-mediated signal transduction, translation, and vesicle-mediated transport (Fig. 3B). This finding is consistent with previous reports [27].

According to our recent publication on the protein interaction network of human colorectal cancer cell-derived extracellular vesicles, vesicular proteins are interconnected via physical interactions and are clustered into functional modules involved in vesicle biogenesis and functions [21]. Figure 4A shows the protein interaction network of 7806 human vesicular proteins from high-throughput proteomes (Supporting Information Table S2). Moreover, 797 common human vesicular proteins are extensively interconnected via physical interactions (Fig. 4B). In addition, the following functional modules are formed by physical and functional interconnections among common human vesicular proteins: cytoskeleton organization (Fig. 4C) and vesicle-mediated transport (Fig. 4D). The vesicle-mediated transport subnetwork is clustered with functional modules of cytoskeleton regulation, endosomal-sorting complexes required for transports, ADP ribosylation factors, SRC signaling, vesicle targeting (including Rab proteins), and exocytosis (Fig. 4D). All common vesicular proteins in these functional modules are known to be involved in the biogenesis and structure of extracellular vesicles [21, 33, 130–134].

4 Transcriptomic analysis of extracellular vesicles

Since the historic discovery of vesicular mRNAs and miRNAs in humans and mice [75–78, 135], extracellular vesicles from various cell types and body fluids have been reported to contain significant quantities of mRNAs and miRNAs [75–100]. Most recent studies have focused on profiling vesicular mRNAs and miRNAs, particularly the horizontal transfer of genetic material that might cause epigenetic reprogramming of recipient cells, and on potential diagnostic indicators of various diseases based on the genetic signature of the originating cell [75–78, 135]. A recent exciting finding is that vesicular mRNAs or miRNAs can be functionally transferred to the target cell, eventually altering gene expression of neighboring and distant cells [75–78, 135]. Direct membrane fusion with the target cell or mixing of the vesicular luminal contents with the recipient cell cytosol after receptor-mediated endocytosis may be involved in this vesicle-mediated horizontal gene transfer [136]. However, the detailed mechanism remains unclear and few studies have been published. Furthermore, several researchers have detected the rRNA,

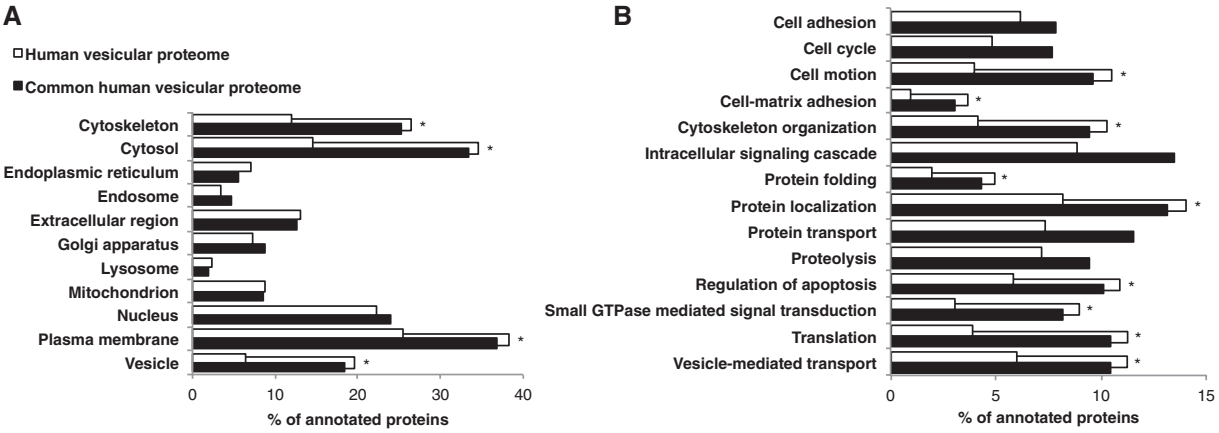

Figure 3. Classification of human vesicular proteins by Gene Ontology cellular component and biological process. The subcellular localization (A) and biological processes (B) of 7806 human vesicular proteins and 797 common human vesicular proteins. Cellular components or biological processes enriched in the common human vesicular proteins were calculated using a modified Fisher's exact test (EASE score) in DAVID (http://david.abcc.ncifcrf.gov) [150]. *$p < 0.001$.

tRNA, and short DNA sequences of retrotransposons in extracellular vesicles [88, 137, 138]. Recent reviews of this subject are available [112, 139, 140].

In total, 44 high-throughput transcriptomic mRNA and miRNA data sets based on microarray and next-generation sequencing have been published to date (Tables 1 and 3) [75–100]. The mRNA contents of extracellular vesicles have various cellular origins, including adipocytes, breast cancer cells, cardiomyocytes, colorectal cancer cells, endothelial cells, gioblastoma cells, hepatocytes, mast cells, mesenchymal stem cells, pancreatic cancer cells, saliva, and serum [75–87]. Advances in miRNA microarray and next-generation sequencing techniques have also generated profiles of the miRNAs present within extracellular vesicles derived from various cell types: e.g., adipocytes, B cells, colorectal cancer cells, dendritic cells, endothelial cells, liver cancer cells, lung cancer cells, gastric cancer cells, macrophages, mast cells, mesenchymal stem cells, monocytes, and T cells [75, 87–96]. In addition, the miRNA contents of extracellular vesicles derived from plasma, saliva, and milk have been reported [97–100].

These high-throughput transcriptomic studies have revealed that a specific subset of mRNAs and miRNAs is selectively incorporated into extracellular vesicles [141]. However, this remains controversial since studies have reported discordant results. Some high-throughput mRNA analyses of extracellular vesicles derived from mast cells, endothelial progenitor cells, and human saliva suggest that only specific mRNA subpopulations are packaged within the extracellular vesicles by means of a selective enrichment process [75, 78, 84]. In contrast, extracellular vesicles derived from glioblastoma and colorectal cancer cells harbor a similar number of mRNAs as the original cells [76, 77]. The latter two studies reported that the mRNA content of extracellular vesicles reflects the cellular mRNA of the originating cell; however, some vesicular RNAs were relatively enriched. Also, whether the specific popula-tions of cellular miRNAs are selectively packaged into extracellular vesicles [75, 77, 89] or not [76, 87, 97, 98] remains disputed.

Many potential reasons exist for the discrepancies among these transcriptomic studies. Because microarray is a very sensitive approach, the presence of contamination, such as nonvesicular RNAs or degraded RNAs, can affect the results. Therefore, the preparation of extracellular vesicles without cellular debris, bovine serum-derived extracellular vesicles, or any other nonvesicular RNA, is critical in vesicular transcriptomic profiling. In addition, the half-life of individual vesicular mRNA or miRNA is unknown, suggesting that rapid purification and appropriate storage of vesicular mRNA are important. Furthermore, mRNA and miRNA are present in extracellular vesicles purified from body fluids, which is rich in RNase [142]. RNase treatment alters the vesicular mRNA profile [84], suggesting the existence of at least two different forms of extracellular RNAs: RNase-sensitive free RNAs and RNase-resistant RNAs that are encapsulated within extracellular vesicles. However, few of the published reports incorporated an RNase-digestion step when purifying vesicular RNA, resulting in the discrepancy in the RNA profile compared with those that did not perform RNase digestion. Therefore, the standard protocols of vesicle purification and high-throughput transcriptomic analysis techniques should include steps designed to reduce the discrepancy among vesicular transcriptomic studies. These studies provide a global view of the sorting mechanisms of RNAs during vesicle formation and vesicular RNA-mediated intercellular communication and may lead to the discovery of disease-specific biomarkers.

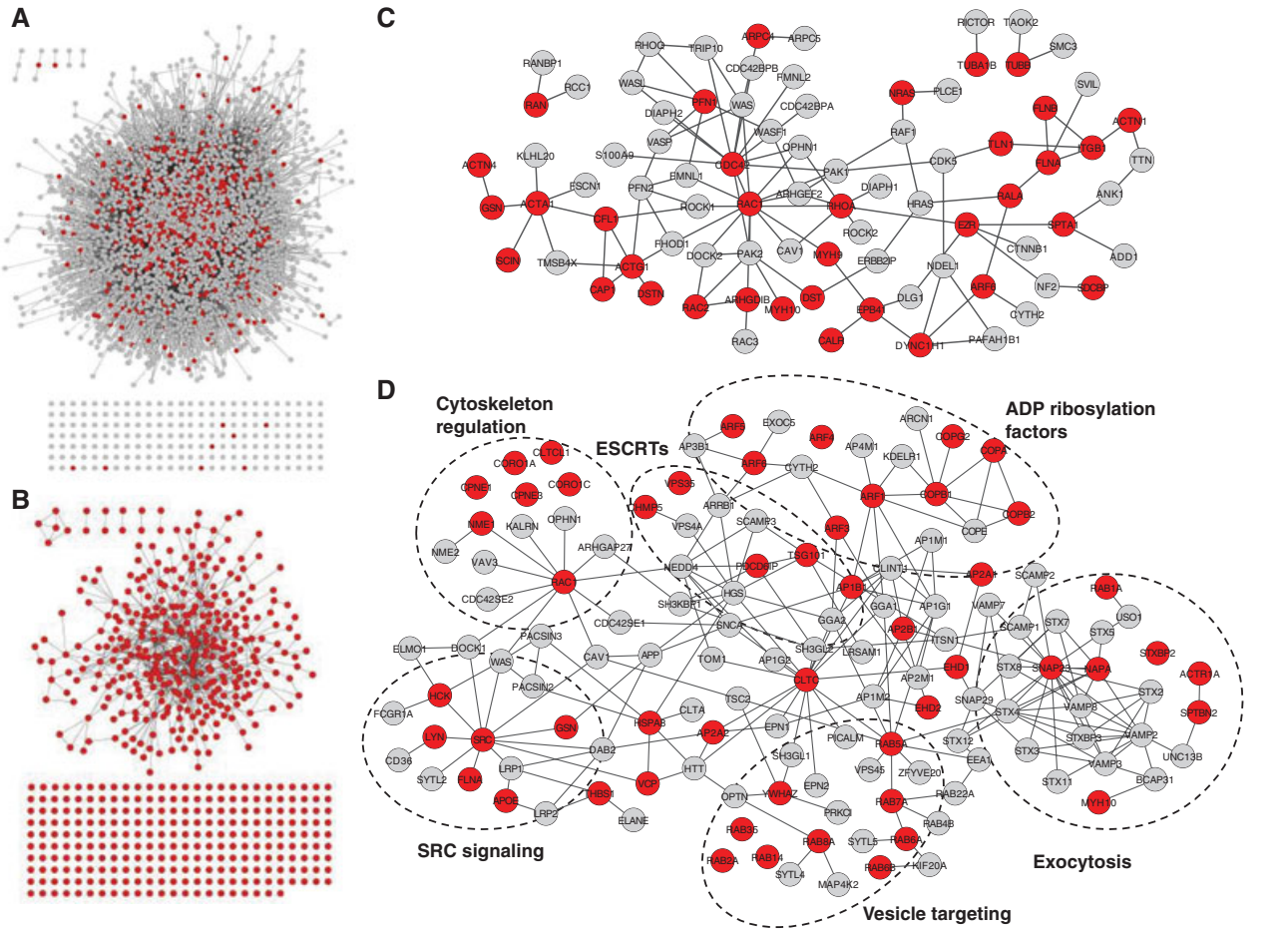

Figure 4. Protein interaction networks of human extracellular vesicles. Protein interaction network of 7806 human vesicular proteins (A) or 797 common human vesicular proteins (B). Common human vesicular proteins are physically and functionally interconnected to form the following functional modules: cytoskeleton organization (C) and vesicle-mediated transport (D). Common and cell type specific human vesicular proteins are indicated by red and gray circles, respectively. ESCRT, endosomal-sorting complexes required for transport.

5 Lipidomic analysis of extracellular vesicles

The list of lipids incorporated in extracellular vesicles has been lengthening for many years. At present, 12 studies provide 15 lipidomic data sets of extracellular vesicles from multiple species, cells, and biological fluids, such as plasma and urine [33, 40, 101–110] (Tables 1 and 4). TLC, LC, GC, and MS are the techniques used most commonly for vesicular lipidomic analysis. Recent reviews of extracellular vesicle lipidomics are available [6, 143].

Although differences in lipid composition have been found in extracellular vesicles derived from different cell types, the vesicular lipid bilayer comprises mainly plasma membrane lipids including phospholipids, sphingomyelin, ganglioside GM3, and cholesterol. When compared with their parent cells, membranes of extracellular vesicles are enriched with phosphatidylserine, disaturated phosphatidylethanolamine, disaturated phosphatidylcholine, sphingomyelin, ganglioside GM3, and cholesterol [6, 101, 102]. These enriched lipids as well as lipid-raft-associated proteins in vesicular membranes provide extracellular vesicles with stability and structural rigidity [106, 144–146].

In 2002, evidence of the biological activity of vesicular lipid was reported for the first time [114]. In this report, Kim et al. reported that the angiogenic activity of tumor-derived extracellular vesicles in vitro and in vivo is mediated mainly by sphingomyelin. In addition, vesicle-bound prostaglandins trigger prostaglandin-dependent intracellular signaling pathways within target cells [105]. Roles for vesicle-bound lysophosphatidylcholine in maturation of dendritic cells and triggering of lymphocyte chemotaxis via the G protein-coupled receptor have also been proposed [6]. Due to the increased local density and overall avidity, membrane-bound prostaglandin or lysophosphatidylcholine on extracellular vesicles exert their biological activity more efficiently than those in the soluble form, as reported for vesicular ICAM-1 protein [147].

Table 3. High-throughput transcriptomic studies on extracellular vesicles

	Species	Isolation strategies	Transcriptomics analysis strategies	Published year	Reference
mRNA					
Cell types					
Endothelial progenitor cell	*Homo sapiens*	Preclearing (2000 × *g*) > Pelleting (100 000 × *g*)	Microarray	2007	[78]
Mast cell (MC/9)[a)]	*Mus musculus*	Preclearing (500 × *g*, 16 500 × *g*) > Filtration (0.22 μm) > Pelleting (120 000 × *g*) > Sucrose density gradient ultracentrifugation (100 000 × *g*) > Pelleting (150 000 × *g*)	Microarray	2007	[75]
Gioblastoma cell	*Homo sapiens*	Preclearing (300 × *g*, 16 500 × *g*) > Filtration (0.22 μm) > Pelleting (110 000 × *g*)	Microarray	2008	[77]
Colorectal cancer cell (SW480)	*Homo sapiens*	Preclearing (500 × *g*, 2000 × *g*) > Concentration (100 kDa) > Sucrose cushion ultracentrifugation (100 000 × *g*) > OptiPrep density gradient ultracentrifugation (200 000 × *g*)	Microarray	2009	[76]
Mesenchymal stem cell	*Homo sapiens*	Preclearing (2000 × *g*) > Pelleting (100 000 × *g*)	Microarray	2009	[82]
Adipocyte (3T3-L1)[a)]	*Mus musculus*	Preclearing (15 000 × *g*) > Pelleting (100 000 × *g*)	Microarray	2010	[87]
Hepatocyte	*Homo sapiens*	Preclearing (2000 × *g*) > Pelleting (100 000 × *g*)	Microarray	2010	[80]
Mast cell (MC/9)	*Mus musculus*	Preclearing (300 × *g*, 16 500 × *g*) > Filtration (0.2 μm) > Pelleting (120 000 × *g*)	Microarray	2010	[81]
Pancreas cancer cell	*Rattus norvegicus*	Preclearing (500 × *g*, 2000 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose gradient ultracentrifugation (150 000 × *g*)	Microarray	2010	[79]
Cardiomyocyte (HL-1)	*Homo sapiens*	Preclearing (300 × *g*, 10 000 × *g*) > Pelleting (130 000 × *g*)	Microarray	2012	[86]
Salivary gland epithelial cell (HSG), breast cancer cell (MDA-MB-231)	*Homo sapiens*	Preclearing (300 × *g*, 2000 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*)	Microarray	2012	[83]
Body fluids					
Saliva	*Homo sapiens*	Preclearing (2600 × *g*, 12 000 × *g*) > Pelleting (120 000 × *g*)	Microarray	2010	[84]
Serum	*Homo sapiens*	Filtration (0.8 μm) > Pelleting (120 000 × *g*)	Microarray	2012	[85]
miRNA					
Cell types					
Mast cell (MC/9)[a)]	*Mus musculus*	Preclearing (500 × *g*, 16 500 × *g*) > Filtration (0.22 μm) > Pelleting (120 000 × *g*) > Sucrose density gradient ultracentrifugation (100 000 × *g*) > Pelleting (150 000 × *g*)	Microarray	2007	[75]
Adipocyte (3T3-L1)[a)]	*Mus musculus*	Preclearing (15 000 × *g*) > Pelleting (100 000 × *g*)	Microarray	2010	[87]
Lung cancer cell (A549), liver cancer cell (HepG2)	*Homo sapiens*	Microvesicle: Preclearing (1000 × *g*) > Pelleting (16 000 × *g*) Exosome: preclearing (1000 × *g*, 16 000 × *g*) > Pelleting (140 000 × *g*)220 Pellet: preclearing (1000 × *g*, 16 000 × *g*, 140 000 × *g*) > Pelleting (220 000 × *g*)	Microarray	2010	[91]

Table 3. Continued

	Species	Isolation strategies	Transcriptomics analysis strategies	Published year	Reference
Lung cancer cell (NCI_H69, DMS563, SBC-3), gastric cancer cell (AZ521, AZP7a), colorectal cancer cell (SW480, SW620)	*Homo sapiens*	Preclearing (800 × *g*) > Filtration (0.1 μm) > Concentration (100 kDa) > Pelleting (100 000 × *g*)	Microarray	2010	[92]
Mesenchymal stem cell (HuES9.E1)	*Homo sapiens*	HPLC	Microarray	2010	[94]
Lung	*Mus musculus*	Pelleting (100 000 × *g*)	Microarray	2010	[96]
B cell (Raji), T cell (Jurkat), dendritic cell	*Homo sapiens*	Preclearing (320 × *g*) > Filtration (0.22 μm) > Pelleting (100 000 × *g*)	Microarray	2011	[89]
Liver cancer cell (Hep3B, PLC/PRF/5)	*Homo sapiens*	Preclearing (200 × *g*) > Pelleting (100 000 × *g*)	Microarray	2011	[88]
Macrophage	*Homo sapiens*	Preclearing (500 × *g*, 16 500 × *g*) > Filtration (0.22 μm) > Pelleting (120 000 × *g*)	Microarray	2011	[93]
Dendritic cell	*Mus musculus*	Preclearing (300 × *g*, 1200 × *g*, 10 000 × *g*) > Filtration (100 kDa) > Sucrose cushion ultracentrifugation (100 000 × *g*)	Microarray	2012	[90]
Monocyte (THP-1), endothelial cell (HUVEC)	*Homo sapiens*	Preclearing (120 × *g*) > Pelleting (16 000 × *g*)	Next-generation sequencing	2012	[95]
Body fluids					
Plasma	*Homo sapiens*	MACS (anti-EpCAM) > Pelleting (100 000 × *g*)	Microarray	2008	[97]
Plasma	*Homo sapiens*	MACS (anti-EpCAM)	Microarray	2009	[98]
Saliva	*Homo sapiens*	Preclearing (1500 × *g*, 17 000 × *g*) > Pelleting (160 000 × *g*)	Microarray	2010	[99]
Breast milk	*Homo sapiens*	Preclearing (2000 × *g*, 12 000 × *g*) > Filtraction (0.45 μm) > ExoQuick	Next-generation sequencing	2012	[100]

a) mRNA and miRNA transcriptomes identified in the same study.
MACS, magnetic cell separation.

Table 4. Lipidomic studies on exosomes and ectosomes

	Species	Isolation strategies	Lipidomic analysis strategies	Published year	Reference
Cell types					
Reticulocyte	*Ovis aries*	Preclearing (8000 × *g*) > Pelleting (100 000 × *g*)	TLC	1987	[109]
Reticulocyte	*Cavia porcellus*	Preclearing (12 000 × *g*) > Pelleting (100 000 × *g*)	TLC	1989	[101]
B cell (RN HLA-DR15$^+$)	*Homo sapiens*	Preclearing (200 × *g*, 500 × *g*, 2000 × *g*, 10 000 × *g*) > Pelleting (70 000 × *g*) > Sucrose gradient ultracentrifugation (270 000 × *g*) > Pelleting (350 000 × *g*)	TLC	2003	[102]
Dendritic cell, mast cell (RBL-2H3)	*Homo sapiens* and *Rattus novergicus*	Preclearing (300 × *g*, 2000 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*)	LC and GC	2004	[103]
Mast cell (RBL-2H3)	*Rattus novergicus*	Preclearing (1550 × *g*) > Pelleting (17 590 × *g*)	LC	2004	[104]
Oligodendroglial precursor cell (Oli-neu)	*Mus musculs*	Preclearing (3000 × *g*, 4000 × *g*, 10 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose gradient ultracentrifugation (200 000 × *g*)	ESI-MS	2008	[33]
Melanoma cell (Mel1)	*Homo sapiens*	Preclearing (300 × *g*, 1200 × *g*, 10 000 × *g*, 70 000 × *g*) > Pelleting (100 000 × *g*) > Sucrose gradient ultracentrifugation (100 000 × *g*) > Pelleting (200 000 × *g*)	TLC	2009	[106]
Mast cell (RBL-2H3)	*Rattus norvegicus*	Preclearing (300 × *g*, 2000 × *g*, 10 000 × *g*) > Pelleting (110 000 × *g*)	TLC	2010	[105]
Reticulocyte	*Rattus norvegicus*	Preclearing (20 000 × *g*) > Pelleting (100 000 × *g*)	TLC	2011	[40]
Paracoccidioides brasiliensis	*Paracoccidioides brasiliensis*	Preclearing (4000 × *g*, 15 000 × *g*) > Concentration (100 kDa) > Preclearing (15 000 × *g*) > Pelleting (100 000 × *g*)	GC-EI-MS	2012	[107]
Body fluids					
Plasma	*Homo sapiens*	Preclearing (1550 × *g*) > Pelleting (17 590 × *g*)	TLC	2002	[108]
Urine	*Homo sapiens*	Preclearing (100 × *g*, 17 000 × *g*) > Pelleting (200 000 × *g*)	LC-ESI-MS/MS	2012	[110]

CI, chemimal ionizaion; EI, electron ionization.

Although lipidomics has gained special interest as a novel field in the biology of extracellular vesicles, only a handful of lipids have been identified to date. In future, application of LC and MS will facilitate profiling of the lipid components of extracellular vesicles derived from various cell types and body fluids. These comprehensive lipidomic studies might help to elucidate the roles of lipids in vesicle biogenesis and their biological functions.

6 Clinical application

Recent studies have drawn attention to the importance of extracellular vesicles in clinical applications as diagnostic and therapeutic tools in human diseases [24–27, 111, 112]. Advances in the proteomic, transcriptomic, and lipidomic studies on extracellular vesicles from various cell types and body fluids have facilitated conceptual advancements in the field of intercellular communication under physiological and pathological conditions. As increasing numbers of vesicular components are identified, vesicle-specific and cell type specific cargos (proteins, mRNAs, miRNAs, and lipids) of the extracellular vesicles represent rich sources of diagnostic biomarkers in various human diseases such as cancer, acute liver injury, and stroke. In addition, extracellular vesicles are easily isolated from various biological fluids (plasma, urine, breast milk, semen, amniotic fluid, saliva, and ascites). More importantly, these fluid-derived extracellular vesicles contain disease-specific proteins, mRNAs, and miRNAs reflecting their originating cell type and status [14–20]. Therefore, screening of extracellular vesicles from various physiological sources may represent a noninvasive method of diagnosis and determination of the prognosis of human various diseases. In addition, the clinical application of extracellular vesicles in immunotherapy of cancer, rheumatoid arthritis, severe acute respiratory syndrome, and transplantation is a

promising area of research. For example, the potential of tumor antigen-loaded native or artificial extracellular vesicles as a cell-free tumor vaccine that promotes T cell-dependent antitumor effects has been investigated [148, 149]. However, additional studies are required to demonstrate their diagnostic and therapeutic potential in human diseases. Recent reviews of this subject, including disease-specific candidate vesicular biomarkers, are available [27, 111, 112].

7 Concluding remarks

In the present review, we focused on the contribution of proteomic, transcriptomic, and lipidomic studies to understanding of the molecular composition of extracellular vesicles derived from various cell types and body fluids. The results indicated that the molecular components of extracellular vesicles are significantly different from those of the parental cells. These vesicle-specific proteins, miRNAs, and lipids are likely linked to the pathophysiological functions of extracellular vesicles and may lead to the discovery of biomarkers and facilitate unraveling of the molecular mechanisms underlying cargo-sorting and biogenesis of extracellular vesicles. However, the study of the biology of extracellular vesicles is in its infancy; many questions remain to be answered. Together with conventional biological research, high-throughput multiomics-based quantitative and comparative analyses of extracellular vesicles will provide new knowledge and aid in comprehension of these evolutionarily conserved extracellular vesicles.

We apologize to colleagues whose work we could not cite owing to length restrictions. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 20120005634).

The authors have declared no conflict of interest.

8 References

[1] Thery, C., Ostrowski, M., Segura, E., Membrane vesicles as conveyors of immune responses. *Nat. Rev. Immunol.* 2009, *9*, 581–593.

[2] Cocucci, E., Racchetti, G., Meldolesi, J., Shedding microvesicles: artefacts no more. *Trends Cell Biol.* 2009, *19*, 43–51.

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

[4] Simpson, R. J., Jensen, S. S., Lim, J. W., Proteomic profiling of exosomes: current perspectives. *Proteomics* 2008, *8*, 4083–4099.

[5] Bellingham, S. A., Guo, B. B., Coleman, B. M., Hill, A. F., Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases?. *Front. Physiol.* 2012, *3*, 124.

[6] Subra, C., Laulagnier, K., Perret, B., Record, M., Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie* 2007, *89*, 205–212.

[7] Lee, E. Y., Choi, D. S., Kim, K. P., Gho, Y. S., Proteomics in Gram-negative bacterial outer membrane vesicles. *Mass Spectrom. Rev.* 2008, *27*, 535–555.

[8] Deatherage, B. L., Cookson, B. T., Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life. *Infect. Immun.* 2012, *80*, 1948–1957.

[9] Lee, E. Y., Choi, D. Y., Kim, D. K., Kim, J. W. et al., Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of *Staphylococcus aureus*-derived membrane vesicles. *Proteomics* 2009, *9*, 5425–5436.

[10] Garcia, B. A., Smalley, D. M., Cho, H., Shabanowitz, J. et al., The platelet microparticle proteome. *J. Proteome Res.* 2005, *4*, 1516–1521.

[11] Miguet, L., Pacaud, K., Felden, C., Hugel, B. et al., Proteomic analysis of malignant lymphocyte membrane microparticles using double ionization coverage optimization. *Proteomics* 2006, *6*, 153–171.

[12] Choi, D. S., Lee, J. M., Park, G. W., Lim, H. W. et al., Proteomic analysis of microvesicles derived from human colorectal cancer cells. *J. Proteome Res.* 2007, *6*, 4646–4655.

[13] Banfi, C., Brioschi, M., Wait, R., Begum, S. et al., Proteome of endothelial cell-derived procoagulant microparticles. *Proteomics* 2005, *5*, 4443–4455.

[14] Caby, M. P., Lankar, D., Vincendeau-Scherrer, C., Raposo, G., Bonnerot, C., Exosomal-like vesicles are present in human blood plasma. *Int. Immunol.* 2005, *17*, 879–887.

[15] Pisitkun, T., Shen, R. F., Knepper, M. A., Identification and proteomic profiling of exosomes in human urine. *Proc. Natl. Acad. Sci. USA* 2004, *101*, 13368–13373.

[16] Admyre, C., Johansson, S. M., Qazi, K. R., Filen, J. J. et al., Exosomes with immune modulatory features are present in human breast milk. *J. Immunol.* 2007, *179*, 1969–1978.

[17] Utleg, A. G., Yi, E. C., Xie, T., Shannon, P. et al., Proteomic analysis of human prostasomes. *Prostate* 2003, *56*, 150–161.

[18] Keller, S., Rupp, C., Stoeck, A., Runz, S. et al., CD24 is a marker of exosomes secreted into urine and amniotic fluid. *Kidney Int.* 2007, *72*, 1095–1102.

[19] Ogawa, Y., Kanai-Azuma, M., Akimoto, Y., Kawakami, H. et al., Exosome-like vesicles with dipeptidyl peptidase IV in human saliva. *Biol. Pharm. Bull.* 2008, *31*, 1059–1062.

[20] Choi, D. S., Park, J. O., Jang, S. C., Yoon, Y. J. et al., Proteomic analysis of microvesicles derived from human colorectal cancer ascites. *Proteomics* 2011, *11*, 2745–2751.

[21] Choi, D. S., Yang, J. S., Choi, E. J., Jang, S. C. et al., The protein interaction network of extracellular vesicles derived from human colorectal cancer cells. *J. Proteome Res.* 2012, *11*, 1144–1151.

[22] Fevrier, B., Vilette, D., Archer, F., Loew, D. et al., Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci. USA* 2004, *101*, 9683–9688.

[23] Gould, S. J., Booth, A. M., Hildreth, J. E., The Trojan exosome hypothesis. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 10592–10597.

[24] Shedden, K., Xie, X. T., Chandaroy, P., Chang, Y. T. et al., Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. *Cancer Res.* 2003, *63*, 4331–4337.

[25] Simpson, R. J., Lim, J. W., Moritz, R. L., Mathivanan, S., Exosomes: proteomic insights and diagnostic potential. *Expert Rev. Proteomics* 2009, *6*, 267–283.

[26] Chaput, N., Taieb, J., Andre, F., Zitvogel, L., The potential of exosomes in immunotherapy. *Expert Opin. Biol. Ther.* 2005, *5*, 737–747.

[27] Raimondo, F., Morosi, L., Chinello, C., Magni, F., Pitto, M., Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery. *Proteomics* 2011, *11*, 709–720.

[28] Gyorgy, B., Szabo, T. G., Pasztoi, M., Pal, Z. et al., Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell. Mol. Life Sci.* 2011, *68*, 2667–2688.

[29] Shen, B., Wu, N., Yang, J. M., Gould, S. J., Protein targeting to exosomes/microvesicles by plasma membrane anchors. *J. Biol. Chem.* 2011, *286*, 14383–14395.

[30] Fang, Y., Wu, N., Gan, X., Yan, W. et al., Higher-order oligomerization targets plasma membrane proteins and HIV gag to exosomes. *PLoS Biol.* 2007, *5*, e158.

[31] Nabhan, J. F., Hu, R., Oh, R. S., Cohen, S. N. et al., Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. *Proc. Natl. Acad. Sci. USA* 2012, *109*, 4146–4151.

[32] Wehman, A. M., Poggioli, C., Schweinsberg, P., Grant, B. D. et al., The P4-ATPase TAT-5 inhibits the budding of extracellular vesicles in *C. elegans* embryos. *Curr. Biol.* 2011, *21*, 1951–1959.

[33] Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L. et al., Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science*, *319*, 1244–1247.

[34] Miguet, L., Bechade, G., Fornecker, L., Zink, E. et al., Proteomic analysis of malignant B-cell derived microparticles reveals CD148 as a potentially useful antigenic biomarker for mantle cell lymphoma diagnosis. *J. Proteome Res.* 2009, *8*, 3346–3354.

[35] Buschow, S. I., van Balkom, B. W., Aalberts, M., Heck, A. J. et al., MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis. *Immunol. Cell Biol.* 2010, *88*, 851–856.

[36] Potolicchio, I., Carven, G. J., Xu, X., Stipp, C. et al., Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. *J. Immunol.* 2005, *175*, 2237–2243.

[37] Bernimoulin, M., Waters, E. K., Foy, M., Steele, B. M. et al., Differential stimulation of monocytic cells results in distinct populations of microparticles. *J. Thromb. Haemost.* 2009, *7*, 1019–1028.

[38] Dean, W. L., Lee, M. J., Cummins, T. D., Schultz, D. J. et al., Proteomic and functional characterisation of platelet microparticle size classes. *Thromb. Haemost.* 2009, *102*, 711–718.

[39] Bosman, G. J., Lasonder, E., Luten, M., Roerdinkholder-Stoelwinder, B. et al., The proteome of red cell membranes and vesicles during storage in blood bank conditions. *Transfusion* 2008, *48*, 827–835.

[40] Carayon, K., Chaoui, K., Ronzier, E., Lazar, I. et al., Proteolipidic composition of exosomes changes during reticulocyte maturation. *J. Biol. Chem.* 2011, *286*, 34426–34439.

[41] Turiak, L., Misjak, P., Szabo, T. G., Aradi, B. et al., Proteomic characterization of thymocyte-derived microvesicles and apoptotic bodies in BALB/c mice. *J. Proteomics* 2011, *74*, 2025–2033.

[42] Kim, H. S., Choi, D. Y., Yun, S. J., Choi, S. M. et al., Proteomic analysis of microvesicles derived from human mesenchymal stem cells. *J. Proteome Res.* 2012, *11*, 839–849.

[43] Kang, D., Oh, S., Ahn, S. M., Lee, B. H., Moon, M. H., Proteomic analysis of exosomes from human neural stem cells by flow field-flow fractionation and nanoflow liquid chromatography-tandem mass spectrometry. *J. Proteome Res.* 2008, *7*, 3475–3480.

[44] Tauro, B. J., Greening, D. W., Mathias, R. A., Ji, H. et al., Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. *Methods* 2012, *56*, 293–304.

[45] Mathivanan, S., Lim, J. W., Tauro, B. J., Ji, H. et al., Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature. *Mol. Cell. Proteomics* 2010, *9*, 197–208.

[46] Aoki, N., Jin-no, S., Nakagawa, Y., Asai, N. et al., Identification and characterization of microvesicles secreted by 3T3-L1 adipocytes: redox- and hormone-dependent induction of milk fat globule-epidermal growth factor 8-associated microvesicles. *Endocrinology* 2007, *148*, 3850–3862.

[47] Rodrigues, M. L., Nakayasu, E. S., Oliveira, D. L., Nimrichter, L. et al., Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. *Eukaryot. Cell* 2008, *7*, 58–67.

[48] Koppen, T., Weckmann, A., Muller, S., Staubach, S. et al., Proteomics analyses of microvesicles released by *Drosophila* Kc167 and S2 cells. *Proteomics* 2011, *11*, 4397–4410.

[49] Peterson, D. B., Sander, T., Kaul, S., Wakim, B. T. et al., Comparative proteomic analysis of PAI-1 and TNF-alpha-derived endothelial microparticles. *Proteomics* 2008, *8*, 2430–2446.

[50] Prokopi, M., Pula, G., Mayr, U., Devue, C. et al., Proteomic analysis reveals presence of platelet microparticles in

endothelial progenitor cell cultures. *Blood* 2009, *114*, 723–732.

[51] Conde-Vancells, J., Rodriguez-Suarez, E., Embade, N., Gil, D. et al., Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. *J. Proteome Res.* 2008, *7*, 5157–5166.

[52] Chavez-Munoz, C., Kilani, R. T., Ghahary, A., Profile of exosomes related proteins released by differentiated and undifferentiated human keratinocytes. *J. Cell. Physiol.* 2009, *221*, 221–231.

[53] Kramer-Albers, E. M., Bretz, N., Tenzer, S., Winterstein, C. et al., Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: trophic support for axons? *Proteomics Clin. Appl.* 2007, *1*, 1446–1461.

[54] Xiao, Z., Camalier, C. E., Nagashima, K., Chan, K. C. et al., Analysis of the extracellular matrix vesicle proteome in mineralizing osteoblasts. *J. Cell. Physiol.* 2007, *210*, 325–335.

[55] Lee, H. S., Jeong, J., Lee, K. J., Characterization of vesicles secreted from insulinoma NIT-1 cells. *J. Proteome Res.* 2009, *8*, 2851–2862.

[56] Oliveira, D. L., Nakayasu, E. S., Joffe, L. S., Guimaraes, A. J. et al., Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. *PloS One* 2010, *5*, e11113.

[57] Stamer, W. D., Hoffman, E. A., Luther, J. M., Hachey, D. L. et al., Protein profile of exosomes from trabecular meshwork cells. *J. Proteomics* 2011, *74*, 796–804.

[58] Atay, S., Gercel-Taylor, C., Kesimer, M., Taylor, D. D., Morphologic and proteomic characterization of exosomes released by cultured extravillous trophoblast cells. *Exp. Cell Res.* 2011, *317*, 1192–1202.

[59] McCann, M., Moore, Z. E., Interventions for preventing infectious complications in haemodialysis patients with central venous catheters. *Cochrane Database Syst. Rev.* 2010, *1*, CD006894.

[60] Gonzalez-Begne, M., Lu, B., Han, X., Hagen, F. K. et al., Proteomic analysis of human parotid gland exosomes by multidimensional protein identification technology (MudPIT). *J. Proteome Res.* 2009, *8*, 1304–1314.

[61] Ochieng, J., Pratap, S., Khatua, A. K., Sakwe, A. M., Anchorage-independent growth of breast carcinoma cells is mediated by serum exosomes. *Exp. Cell Res.* 2009, *315*, 1875–1888.

[62] Reinhardt, T. A., Lippolis, J. D., Nonnecke, B. J., Sacco, R. E., Bovine milk exosome proteome. *J. Proteomics* 2012, *75*, 1486–1492.

[63] Looze, C., Yui, D., Leung, L., Ingham, M. et al., Proteomic profiling of human plasma exosomes identifies PPARgamma as an exosome-associated protein. *Biochem. Biophys. Res. Commun.* 2009, *378*, 433–438.

[64] Ramacciotti, E., Hawley, A. E., Wrobleski, S. K., Myers, D. D., Jr. et al., Proteomics of microparticles after deep venous thrombosis. *Thromb. Res.* 2010, *125*, e269–e274.

[65] Watts, J. A., Lee, Y. Y., Gellar, M. A., Fulkerson, M. B. et al., Proteomics of microparticles after experimental pulmonary embolism. *Thromb. Res.* 2012, *130*, 122–128.

[66] Xiao, H., Wong, D. T., Proteomic analysis of microvesicles in human saliva by gel electrophoresis with liquid chromatography-mass spectrometry. *Anal. Chim. Acta* 2012, *723*, 61–67.

[67] Poliakov, A., Spilman, M., Dokland, T., Amling, C. L. et al., Structural heterogeneity and protein composition of exosome-like vesicles (prostasomes) in human semen. *Prostate* 2009, *69*, 159–167.

[68] Smalley, D. M., Sheman, N. E., Nelson, K., Theodorescu, D., Isolation and identification of potential urinary microparticle biomarkers of bladder cancer. *J. Proteome Res.* 2008, *7*, 2088–2096.

[69] Gonzales, P. A., Pisitkun, T., Hoffert, J. D., Tchapyjnikov, D. et al., Large-scale proteomics and phosphoproteomics of urinary exosomes. *J. Am. Soc. Nephrol.* 2009, *20*, 363–379.

[70] Hogan, M. C., Manganelli, L., Woollard, J. R., Masyuk, A. I. et al., Characterization of PKD protein-positive exosome-like vesicles. *J. Am. Soc. Nephrol.* 2009, *20*, 278–288.

[71] Conde-Vancells, J., Rodriguez-Suarez, E., Gonzalez, E., Berisa, A. et al., Candidate biomarkers in exosome-like vesicles purified from rat and mouse urine samples. *Proteomics Clin. Appl.* 2010, *4*, 416–425.

[72] Moon, P. G., Lee, J. E., You, S., Kim, T. K. et al., Proteomic analysis of urinary exosomes from patients of early IgA nephropathy and thin basement membrane nephropathy. *Proteomics* 2011, *11*, 2459–2475.

[73] Wang, Z., Hill, S., Luther, J. M., Hachey, D. L. et al., Proteomic analysis of urine exosomes by multidimensional protein identification technology (MudPIT). *Proteomics* 2012, *12*, 329–338.

[74] Choi, D. S., Choi, D. Y., Hong, B. S., Jang, S. C. et al., Quantitative proteomics of extracellular vesicles derived from human primary and metastatic colorectal cancer cells. *J. Extracellular Vesicles* 2012, *1*, 18704.

[75] Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M. et al., Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell. Biol.* 2007, *9*, 654–659.

[76] Hong, B. S., Cho, J. H., Kim, H., Choi, E. J. et al., Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BMC Genomics* 2009, *10*, 556.

[77] Skog, J., Wurdinger, T., van Rijn, S., Meijer, D. H. et al., Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell. Biol.* 2008, *10*, 1470–1476.

[78] Deregibus, M. C., Cantaluppi, V., Calogero, R., Lo Iacono, M. et al., Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* 2007, *110*, 2440–2448.

[79] Nazarenko, I., Rana, S., Baumann, A., McAlear, J. et al., Cell surface tetraspanin Tspan8 contributes to molecular pathways of exosome-induced endothelial cell activation. *Cancer Res.* 2010, *70*, 1668–1678.

[80] Herrera, M. B., Fonsato, V., Gatti, S., Deregibus, M. C. et al., Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats. *J. Cell. Mol. Med.* 2010, *14*, 1605–1618.

[81] Eldh, M., Ekstrom, K., Valadi, H., Sjostrand, M. et al., Exosomes communicate protective messages during oxidative stress; possible role of exosomal shuttle RNA. *PloS One* 2010, *5*, e15353.

[82] Bruno, S., Grange, C., Deregibus, M. C., Calogero, R. A. et al., Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J. Am. Soc. Nephrol.* 2009, *20*, 1053–1067.

[83] Lau, C. S., Wong, D. T., Breast cancer exosome-like microvesicles and salivary gland cells interplay alters salivary gland cell-derived exosome-like microvesicles in vitro. *PloS One* 2012, *7*, e33037.

[84] Palanisamy, V., Sharma, S., Deshpande, A., Zhou, H. et al., Nanostructural and transcriptomic analyses of human saliva derived exosomes. *PloS One* 2010, *5*, e8577.

[85] Noerholm, M., Balaj, L., Limperg, T., Salehi, A. et al., RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls. *BMC Cancer* 2012, *12*, 22.

[86] Waldenstrom, A., Genneback, N., Hellman, U., Ronquist, G., Cardiomyocyte microvesicles contain DNA/RNA and convey biological messages to target cells. *PloS One* 2012, *7*, e34653.

[87] Ogawa, R., Tanaka, C., Sato, M., Nagasaki, H. et al., Adipocyte-derived microvesicles contain RNA that is transported into macrophages and might be secreted into blood circulation. *Biochem. Biophys. Res. Commun.* 2010, *398*, 723–729.

[88] Kogure, T., Lin, W. L., Yan, I. K., Braconi, C., Patel, T., Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology* 2011, *54*, 1237–1248.

[89] Mittelbrunn, M., Gutierrez-Vazquez, C., Villarroya-Beltri, C., Gonzalez, S. et al., Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat. Commun.* 2011, *2*, 282.

[90] Montecalvo, A., Larregina, A. T., Shufesky, W. J., Stolz, D. B. et al., Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* 2012, *119*, 756–766.

[91] Wang, K., Zhang, S., Weber, J., Baxter, D. et al., Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res.* 2010, *38*, 7248–7259.

[92] Ohshima, K., Inoue, K., Fujiwara, A., Hatakeyama, K. et al., Let-7 microRNA family is selectively secreted into the extracellular environment via exosomes in a metastatic gastric cancer cell line. *PloS One* 2010, *5*, e13247.

[93] Yang, M., Chen, J., Su, F., Yu, B., et al., Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol. Cancer* 2011, *10*, 117.

[94] Chen, T. S., Lai, R. C., Lee, M. M., Choo, A. B. et al., Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. *Nucleic Acids Res.* 2010, *38*, 215–224.

[95] Diehl, P., Fricke, A., Sander, L., Stamm, J. et al., Microparticles: major transport vehicles for distinct microRNAs in circulation. *Cardiovasc. Res.* 2012, *93*, 633–644.

[96] Aliotta, J. M., Pereira, M., Johnson, K. W., de Paz, N. et al., Microvesicle entry into marrow cells mediates tissue-specific changes in mRNA by direct delivery of mRNA and induction of transcription. *Exp. Hematol.* 2010, *38*, 233–245.

[97] Taylor, D. D., Gercel-Taylor, C., MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol. Oncol.* 2008, *110*, 13–21.

[98] Rabinowits, G., Gercel-Taylor, C., Day, J. M., Taylor, D. D. et al., Exosomal microRNA: a diagnostic marker for lung cancer. *Clin. Lung Cancer* 2009, *10*, 42–46.

[99] Michael, A., Bajracharya, S. D., Yuen, P. S., Zhou, H. et al., Exosomes from human saliva as a source of microRNA biomarkers. *Oral Dis.* 2010, *16*, 34–38.

[100] Zhou, Q., Li, M., Wang, X., Li, Q. et al., Immune-related microRNAs are abundant in breast milk exosomes. *Int. J. Biol. Sci.* 2012, *8*, 118–123.

[101] Vidal, M., Sainte-Marie, J., Philippot, J. R., Bienvenue, A., Asymmetric distribution of phospholipids in the membrane of vesicles released during in vitro maturation of guinea pig reticulocytes: evidence precluding a role for "aminophospholipid translocase." *J. Cell. Physiol.* 1989, *140*, 455–462.

[102] Wubbolts, R., Leckie, R. S., Veenhuizen, P. T., Schwarzmann, G. 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.

[103] Laulagnier, K., Motta, C., Hamdi, S., Roy, S. 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.

[104] Laulagnier, K., Grand, D., Dujardin, A., Hamdi, S. et al., PLD2 is enriched on exosomes and its activity is correlated to the release of exosomes. *FEBS Lett.* 2004, *572*, 11–14.

[105] Subra, C., Grand, D., Laulagnier, K., Stella, A. et al., Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. *J. Lipid Res.* 2010, *51*, 2105–2120.

[106] Parolini, I., Federici, C., Raggi, C., Lugini, L. et al., Microenvironmental pH is a key factor for exosome traffic in tumor cells. *J. Biol. Chem.* 2009, *284*, 34211–34222.

[107] Vallejo, M. C., Nakayasu, E. S., Longo, L. V., Ganiko, L. et al., Lipidomic analysis of extracellular vesicles from the pathogenic phase of *Paracoccidioides brasiliensis*. *PloS One* 2012, *7*, e39463.

[108] Weerheim, A. M., Kolb, A. M., Sturk, A., Nieuwland, R., Phospholipid composition of cell-derived microparticles determined by one-dimensional high-performance thin-layer chromatography. *Anal. Biochem.* 2002, *302*, 191–198.

[109] Johnstone, R. M., Adam, M., Hammond, J. R., 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.

[110] Del Boccio, P., Raimondo, F., Pieragostino, D., Morosi, L. et al., A hyphenated microLC-Q-TOF-MS platform for exosomal lipidomics investigations: application to RCC urinary exosomes. *Electrophoresis* 2012, *33*, 689–696.

[111] D'Souza-Schorey, C., Clancy, J. W., Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers. *Genes Dev.* 2012, *26*, 1287–1299.

[112] Hu, G., Drescher, K. M., Chen, X. M., Exosomal miRNAs: biological properties and therapeutic potential. *Front. Genet.* 2012, *3*, 56.

[113] Wysoczynski, M., Ratajczak, M. Z., Lung cancer secreted microvesicles: underappreciated modulators of microenvironment in expanding tumors. *Int. J. Cancer* 2009, *125*, 1595–1603.

[114] Kim, C. W., Lee, H. M., Lee, T. H., Kang, C. et al., Extracellular membrane vesicles from tumor cells promote angiogenesis via sphingomyelin. *Cancer Res.* 2002, *62*, 6312–6317.

[115] Millimaggi, D., Mari, M., D'Ascenzo, S., Carosa, E. et al., Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells. *Neoplasia* 2007, *9*, 349–357.

[116] Al-Nedawi, K., Meehan, B., Kerbel, R. S., Allison, A. C. et al., Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. *Proc. Natl. Acad. Sci. USA* 2009, *106*, 3794–3799.

[117] Lee, T. H., D'Asti, E., Magnus, N., Al-Nedawi, K. et al., Microvesicles as mediators of intercellular communication in cancer—the emerging science of cellular 'debris'. *Semin. Immunopathol.* 2011, *33*, 455–467.

[118] Corcoran, C., Friel, A. M., Duffy, M. J., Crown, J. et al., Intracellular and extracellular microRNAs in breast cancer. *Clin. Chem.* 2011, *57*, 18–32.

[119] Umezu, T., Ohyashiki, K., Kuroda, M., Ohyashiki, J. H., Leukemia cell to endothelial cell communication via exosomal miRNAs. *Oncogene* 2012, doi: 10.1038/onc.2012.295.

[120] Mathivanan, S., Fahner, C. J., Reid, G. E., Simpson, R. J., ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic Acids Res.* 2012, *40*, D1241–D1244.

[121] Escola, J. M., Kleijmeer, M. J., Stoorvogel, W., Griffith, J. M. et al., Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. *J. Biol. Chem.* 1998, *273*, 20121–20127.

[122] Heijnen, H. F., Schiel, A. E., Fijnheer, R., Geuze, H. J. et al., Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood* 1999, *94*, 3791–3799.

[123] de Gassart, A., Geminard, C., Fevrier, B., Raposo, G. et al., Lipid raft-associated protein sorting in exosomes. *Blood* 2003, *102*, 4336–4344.

[124] Thery, C., Regnault, A., Garin, J., Wolfers, J. et al., Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. *J. Cell. Biol.* 1999, *147*, 599–610.

[125] Thery, C., Boussac, M., Veron, P., Ricciardi-Castagnoli, P. et al., Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. *J. Immunol.* 2001, *166*, 7309–7318.

[126] van Niel, G., Raposo, G., Candalh, C., Boussac, M. et al., Intestinal epithelial cells secrete exosome-like vesicles. *Gastroenterology* 2001, *121*, 337–349.

[127] Skokos, D., Le Panse, S., Villa, I., Rousselle, J. C. et al., Mast cell-dependent B and T lymphocyte activation is mediated by the secretion of immunologically active exosomes. *J. Immunol.* 2001, *166*, 868–876.

[128] Moon, P. G., You, S., Lee, J. E., Hwang, D. et al., Urinary exosomes and proteomics. *Mass. Spectrom. Rev.* 2011, *30*, 1185–1202.

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

[130] Muralidharan-Chari, V., Clancy, J. W., Sedgwick, A., D'Souza-Schorey, C., Microvesicles: mediators of extracellular communication during cancer progression. *J. Cell. Sci.* 2010, *123*, 1603–1611.

[131] Williams, R. L., Urbe, S., The emerging shape of the ESCRT machinery. *Nat. Rev. Mol. Cell. Biol.* 2007, *8*, 355–368.

[132] Muralidharan-Chari, V., Clancy, J., Plou, C., Romao, M. et al., ARF6-regulated shedding of tumor cell-derived plasma membrane microvesicles. *Curr. Biol.* 2009, *19*, 1875–1885.

[133] Ostrowski, M., Carmo, N. B., Krumeich, S., Fanget, I. et al., Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell. Biol.* 2009, *12*, 19–30.

[134] Baietti, M. F., Zhang, Z., Mortier, E., Melchior, A. et al., Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat. Cell. Biol.* 2012, *14*, 677–685.

[135] Ratajczak, J., Miekus, K., Kucia, M., Zhang, J. et al., Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. *Leukemia* 2006, *20*, 847–856.

[136] Mause, S. F., Weber, C., Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ. Res.* 2010, *107*, 1047–1057.

[137] Gibbings, D. J., Ciaudo, C., Erhardt, M., Voinnet, O., Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat. Cell. Biol.* 2009, *11*, 1143–1149.

[138] Balaj, L., Lessard, R., Dai, L., Cho, Y. J. et al., Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nat. Commun.* 2011, *2*, 180.

[139] Henderson, M. C., Azorsa, D. O., The genomic and proteomic content of cancer cell-derived exosomes. *Front. Oncol.* 2012, *2*, 38.

[140] Pant, S., Hilton, H., Burczynski, M. E., The multifaceted exosome: biogenesis, role in normal and aberrant cellular function, and frontiers for pharmacological and biomarker opportunities. *Biochem. Pharmacol.* 2012, *83*, 1484–1494.

[141] Mittelbrunn, M., Sanchez-Madrid, F., Intercellular communication: diverse structures for exchange of genetic information. *Nat. Rev. Mol. Cell. Biol.* 2012, *13*, 328–335.

[142] Kosaka, N., Iguchi, H., Ochiya, T., Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci.* 2010, *101*, 2087–2092.

[143] Kooijmans, S. A., Vader, P., van Dommelen, S. M., van Solinge, W. W. et al., Exosome mimetics: a novel class of drug delivery systems. *Int. J. Nanomedicine* 2012, *7*, 1525–1541.

[144] Needham, D., Nunn, R. S., Elastic deformation and failure of lipid bilayer membranes containing cholesterol. *Biophys. J.* 1990, *58*, 997–1009.

[145] Ramstedt, B., Slotte, J. P., Membrane properties of sphingomyelins. *FEBS Lett.* 2002, *531*, 33–37.

[146] Abi-Rizk, G., Besson, F., Interactions of Triton X-100 with sphingomyelin and phosphatidylcholine monolayers: influence of the cholesterol content. *Colloids Surf. B Biointerfaces* 2008, *66*, 163–167.

[147] Lee, H. M., Choi, E. J., Kim, J. H., Kim, T. D. et al., A membranous form of ICAM-1 on exosomes efficiently blocks leukocyte adhesion to activated endothelial cells. *Biochem. Biophys. Res. Commun.* 2010, *397*, 251–256.

[148] Viaud, S., Ploix, S., Lapierre, V., Thery, C. et al., Updated technology to produce highly immunogenic dendritic cell-derived exosomes of clinical grade: a critical role of interferon-gamma. *J. Immunother.* 2011, *34*, 65–75.

[149] Lee, E. Y., Park, K. S., Yoon, Y. J., Lee, J. et al., Therapeutic effects of autologous tumor-derived nanovesicles on melanoma growth and metastasis. *PLoS One* 2012, *7*, e33330.

[150] Huang da, W., Sherman, B. T., Lempicki, R. A., Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 2009, *4*, 44–57.

