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# Biological Function of Exosomes as Diagnostic Markers and Therapeutic Delivery Vehicles in Carcinogenesis and Infectious Diseases

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## Abstract

Exosomes are nano-sized vesicles that are formed during inward budding of multivesicular bodies and the maturation of endosomes. They are secreted by almost all cell types under normal, pathological, and physiological conditions. They are found in mostly all biological fluids, such as breast milk, blood, urine, and semen. Exosomes are involved in cell-to-cell communication through the biological transfer of lipids, proteins, DNAs, RNAs, mRNAs, and miRNAs. Exosomes are enriched in tetraspanins, enzymes, heat shock proteins, and membrane trafficking proteins. There are numerous techniques that are used to isolate, purify, and characterize exosomes from biofluids. Isolation/purification techniques include ultracentrifugation, filtration, sucrose density gradient centrifugation, etc. Characterization techniques include flow cytometry, electron microscopy, NanoSight tracking analysis, Western blot, etc. These techniques are often used to help principal investigators understand the properties and biological functions of exosomes. However, some of these techniques can be very complicated and challenging, resulting in various drawbacks. Exosomes can be used as potential carriers for therapeutics. Thus, they can serve as biomarkers to diagnosis various diseases that are associated with cancer, genetics, viruses, bacteria, parasites, etc. Therefore, with advances in science and technology, many innovative techniques have been established to exploit the biological properties of exosomes.

**Keywords:** exosome, extracellular vesicles, biogenesis, therapeutics, cancer, infectious diseases, drug delivery

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## 1. The discovery of exosomes

In the early 1980s, researchers Pan, Stahl, and Johnstone discovered a complex mode of extracellular vesicle (EV) secretion while studying the loss of transferrin during the maturation of reticulocytes in blood [1–4]. EVs were believed to bud directly from plasma membrane fragments that were isolated from cultured cells and human bodily fluids [1, 2, 5–7]. The research group showed that small vesicles were formed by inward budding inside an intracellular endosome which lead to the formation of multivesicular bodies (MVBs) [1, 8–10]. The MVBs produce intraluminal vesicles (ILVs) and fuse with the plasma membrane, releasing their contents into the extracellular environment [1, 8, 9]. The ILVs were termed “exosomes” in the late 1980s by Johnstone [2, 9]. Since their discovery approximately 40 years ago [3, 4, 8], exosomes have gained tremendous attention due to their involvement in intercellular communication [11]. EVs were originally believed to be waste products of the cell [8, 12, 13]. We currently recognize EVs as much more.

## 2. Exosome biogenesis and secretion

Exosomes are generated in the endosomal membrane when the ILVs of MVBs are formed during the maturation of early and late endosomes [1]. During maturation, MVBs are fated for lysosomal degradation or fused with the plasma membrane which leads to the secretion of ILVs as exosomes [1, 14]. The generation of the ILVs in MVBs contains the lateral segregation of cargo at the endosomal limiting membrane [15, 16]. In addition, it involves the formation of an inward budding vesicle and the release in the endosomal lumen of the membrane vesicle containing a small portion of cytosol [15, 16].

The Endosomal Sorting Complex Responsible for Transport (ESCRT) mediates exosome biogenesis [1, 17–19]. ESCRTs consist of approximately 20 proteins that are divided into the ESCRT-0, -I, -II, and -III complexes [20, 21]. These complexes contain ubiquitin-binding subunits [18, 21, 22]. The ESCRT-0 complex identifies and sequentially binds to ubiquitylated proteins in the endosomal membrane [23]. The ESCRT-I and -II complexes are responsible for membrane deformation into buds with sequential cargo [21]. The ESCRT-III complex drives vesicle scission [21, 24].

ESCRT-0 contains the hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) protein [22]. HRS identifies ubiquitylated cargo proteins and other constituents in a complex that consist of clathrin, the epidermal growth factor receptor pathway substrate 15 gene, and signal-transducing adaptor molecule [18, 25]. Most importantly, HRS recruit tumor susceptibility gene 101 of the ESCRT-I complex [26]. ESCRT-I is then involved in the recruitment of ESCRT-III, through ESCRT-II or the ESCRT-accessory protein ALG-2 interacting protein-X (Alix) [26]. Lastly, the separation and recycling of the ESCRT machinery interacts with the AAA-ATPase vacuolar protein sorting 4 [19, 27].

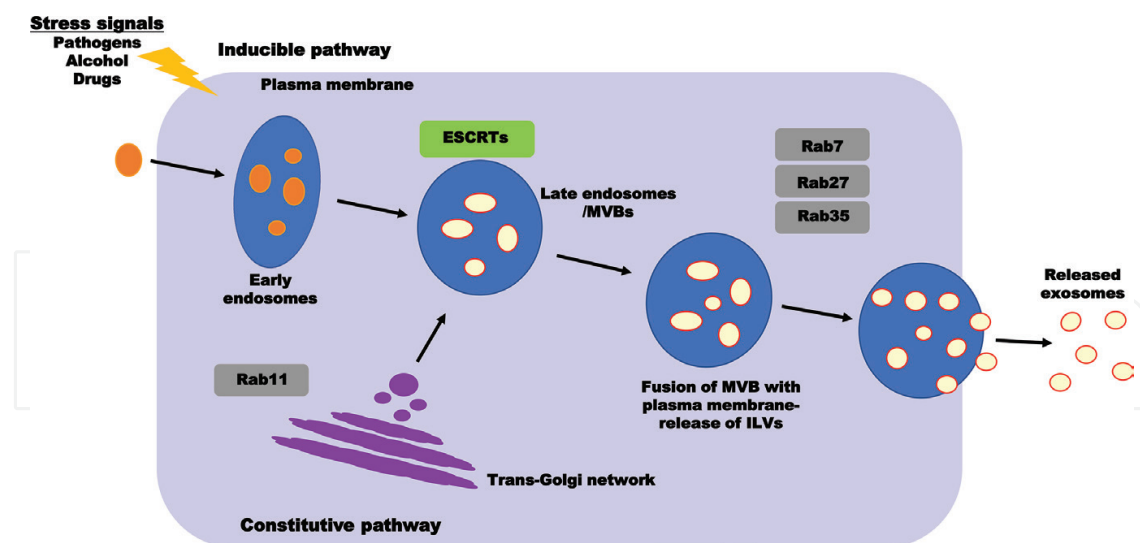
Exosomes are secreted by many cell types during normal, physiological, and pathological conditions [14, 28, 29]. They are secreted from cancer cells [28, 30], platelets [31], neurons

[32], epithelial cells [28, 33, 34], dendritic cells [28, 35], B and T cells [28, 36], astrocytes [28, 37], endothelial cells [28, 38], mast cells [31, 39], and mesenchymal stem cells [28, 40]. Also, exosomes have been identified in most bodily fluids, such as nasal secretion [28, 41], blood [42], serum [28, 43], ascites [44], amniotic fluid [44], urine [28, 45], breast milk [28, 46], and saliva [28, 43].

Depending on the cell type, exosomes are mainly secreted by the constitutive release pathway and/or inducible release pathway [28, 47–51]. In the constitutive secretion pathway, proteins are sorted into vesicles in the Golgi and transported to the cell surface where they fuse with the plasma membrane via exocytosis. In addition, Rab guanosine triphosphatases (GTPases) [52, 53], heterotrimeric G-protein [52], protein kinase D [52, 54], glycosphingolipids, and flotillin [52] are involved in this pathway. Specifically, several Rab GTPases have been shown to act as key regulators of the exosome secretory pathway [49]. Rabs are a large group of small GTPases that regulate protein transport via endocytic and exocytic pathways in all cell types [52, 55]. In addition, Rabs are involved in membrane trafficking (i.e. vesicle budding, membrane fusion, and the transport of vesicles along actin and tubulin) [53]. Rab GTPases are composed of approximately 70 distinct proteins [56, 57]. Common Rab proteins include Rab11, Rab27, and Rab35 [58, 59]. These proteins are all involved in the transport of endolysosomal vesicles toward the plasma membrane [60].

Rab11 was the first Rab GTPase reported study that involved exosome secretion in human leukemic K562 cells by Savina et al. [61, 62]. Specifically, Rab11 is involved in the recycling from an endosomal compartment to the plasma membrane [63–65]. Both Rab27a and Rab27b function in MVE docking at the plasma membrane in several cancer cell lines *in vivo* and *in vitro* [49, 55, 66]. Rab35 mediates MVB docking or tethering in oligodendroglia cells as reported by Hsu et al. [58, 61]. They revealed that the inhibition of Rab35 leads to intracellular accumulation of endosomal vesicles and impairs exosome secretion [58, 61]. In addition, Hsu et al. showed that Rab35 localizes to the surface of oligodendroglia in a GTP-dependent manner, where it regulates vesicular density [58, 61].

Inducible secretion is regulated by many cellular processes [67–69]. This pathway is regulated by stimuli, such as heat shock, hypoxia [52], DNA damage [52, 67, 70], increased intracellular calcium release [52, 67, 70], thrombin [52], extracellular ATP [52, 67], and lipopolysaccharide 39 stimulation [35, 52]. In 2012, King et al. demonstrated that the release of exosomes in breast cancer cells is promoted by hypoxia [71]. In addition, they demonstrated the hypoxic response could potentially be mediated by the hypoxia-inducible factor-1 $\alpha$ —a group of transcription factors that are targeted for degradation under normal oxygen conditions by the action of specific O<sub>2</sub>–, iron- and 2-oxoglutarate dependent prolyl hydroxylases [71, 72]. Another study was reported by Hooper and colleagues in 2012 [73]. In their study, they investigated the inducible release of exosomes cultured from rat microglia cells treated with recombinant carrier-free Wnt3a protein— a family of cysteine rich glycoproteins that play a role in tumorigenesis and act as morphogens during development [69, 73]. They observed that these Wnt3-induced cells increased exosome secretion through a glycogen synthase kinase 3-independent mechanism [73]. The process of exosome biogenesis and secretion is summarized in **Figure 1**.

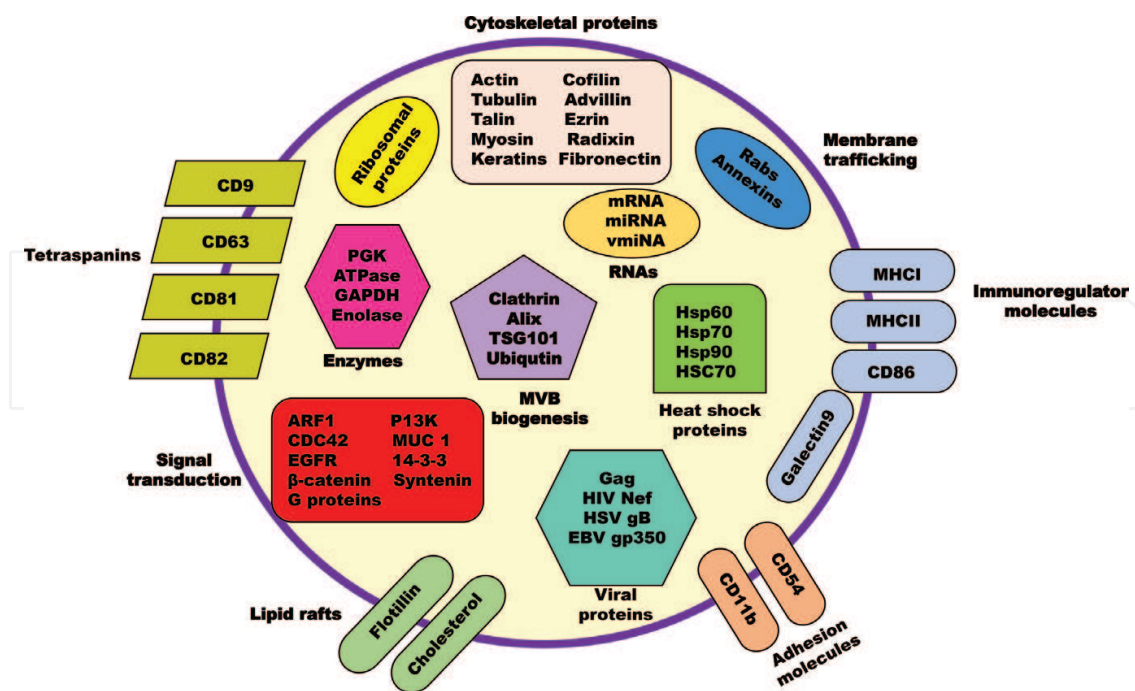


**Figure 1.** Biogenesis and secretion of exosomes. Exosome biogenesis and secretion is a complex process. Exosome secretion can occur by two different mechanisms, constitutive or inducible secretion. One or both of these pathways maybe operational depending on the condition of the cell. Constitutive exosome secretion occurs in various cell types under normal physiological and pathological conditions. Inducible exosome secretion is regulated by stressors (e.g. pathogens, alcohol, drugs). Multivesicular bodies (MBVs), intraluminal vesicles (ILVs), Trans-Golgi network and the Endosomal Sorting Complex Responsible for Transport (ESCRT) are four important compartments involved in exosome biogenesis and secretion. Rab guanosine triphosphatases (GTPases) (7, 11, 27, 35 etc.) are also depicted, they play an important role in exosome secretion.

### 3. Composition of exosomes

Exosomes carry a group of specific proteins, lipids, RNA, microRNA (miRNA), and DNAs, that represents their cells of origin [28, 56, 74], as depicted in **Figure 2**. Recent studies have shown that exosomes contain approximately 194 lipids, 4563 proteins, 1639 messenger RNAs (mRNAs), and 764 miRNAs [28, 75–77]. Exosomes are enriched in molecules, such as the major histocompatibility molecules (MHC) class I and II that play a key role in immunoregulation by processing antigenic peptides [28, 78]. Also, exosomes contain tetraspanins that serve as unique markers [79]. Tetraspanins include: cluster of differentiation (CD) 9, CD63, CD81, and CD82, as well as adhesion molecules CD54 and CD11b [26, 28, 78]. In addition, exosomes are enriched with heat shock proteins (hsps) which act as chaperones and play a key role in cellular responses that are associated with environmental stress. Hsps assist with protein folding and trafficking. Common exosomal proteins include Hsp60, Hsp70, Hsp90, and heat shock protein cognate 70 [16, 78]. Along with tetraspanins and hsps, exosomes contain cytoplasmic proteins such as Rabs and annexins [26, 61]. These proteins promote the fusion of MVB with the cell membrane and the removal of exosomes. Clathrin, Alix, Tumor susceptibility gene 101 (TSG) 101, and ubiquitin are exosomal constituents that are involved in the biogenesis of MVBs [78]. Enzymes that makeup the composition of exosomes consist of protein kinase G (PKG), ATPase, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and enolase. Signal transduction proteins, ADP-ribosylation factor (ARF) 1, cell division control protein 42 (CDC42), epidermal growth factor receptor (EGFR),  $\beta$ -catenin, guanine nucleotide-binding G proteins (G proteins), phosphatidylinositol 3-kinase (P13K), mucin 1 (MUC 1), 14-3-3, and syntenin [68, 78, 80]. Viral proteins, such as group-specific antigen (Gag), Human immunodeficiency virus negative regulatory factor (HIV Nef), Herpes simplex virus glycoprotein B





**Figure 2.** Composition of exosomes. Proteomic, Biochemical, and Immunological investigations have identified many specific proteins and RNAs present in some exosomes. This is a limited representation of common molecules present within some exosomes. Molecules illustrated here are grouped based on category function or protein class: Tetraspanins, Cytoskeletal proteins, Membrane trafficking proteins, Immunoregulator molecules, Adhesion molecules, Lipid rafts, Signal transduction molecules, viral proteins and RNAs.

(HSV gB), Epstein–Barr virus (EBV) gp350 also make up the composition of some exosomes [78, 81]. Exosomes that contain RNA can serve as an alternate pathway of cellular communication [82, 83]. Thus, mRNAs that are found in exosomes can be transferred to target cells and translated into proteins [84]. miRNAs, such as miR-1, miR-15, miR-16, miR-151, miR-375, and lethal-7 play a role in hematopoiesis, exocytosis, tumorigenesis, and angiogenesis [28, 85, 86].

## 4. Exosome isolation/purification methods

Exosomes are isolated from a wide spectrum of biological fluids [87, 88]. To examine the quality of isolated exosomes, numerous methods have been developed to examine and measure their morphology, composition, quantity, and size distribution [88, 89]. With advances in science and technology, many innovative techniques have been established to exploit a specific trait of exosomes, such as their size, shape, density, and surface proteins to aid in their isolation and purification [88, 90]. However, each method has advantages and disadvantages as shown in Table 1.

### 4.1. Ultracentrifugation and filtration-based exosome isolation

Ultracentrifugation is a centrifugation process used for generating acceleration up to  $100,000 \times g$  (approx.  $9800 \text{ km/s}^2$ ) [88]. Differential ultracentrifugation is often used to isolate exosomes [88, 91]. The isolation of exosomes by differential ultracentrifugation contains numerous centrifugation steps, which uses centrifugal force to get rid of residual cells, cellular

Isolation/purification methods	Mechanism	Advantages	Disadvantages
Differential ultracentrifugation [79, 91]	Remove residual cells, large vesicles, and cellular debris; precipitate exosomes [79]	Standard method used to isolate exosomes from cultured media and biological fluids [79, 91]	Effectiveness of the method is lower when biological fluids are used for analysis [79] co-precipitation of protein aggregates, apoptotic bodies, or nucleosomal fragments, which may lead to less sample purity and less correctly bound proteins [91]
Sucrose gradient centrifugation [91]	Separate vesicles based on their different flotation densities [91, 97]	Allows separation of the low-density exosomes from other vesicles, particles and contaminants [91]	Cannot separate exosomes from viruses because of their similarities in density and size [91]
Filtration [79, 91]	Used to separate exosomes from proteins and other macroparticles using ultrafiltration membranes [79]	Allows separation of soluble molecules and small particles from exosomes [79]	Loss of analysis due to adhesion. Contamination of isolated EVs. Exosomes can potentially be deformed or damaged due to additional force being applied pass the analyzed liquid through the membrane [79, 91]
Size exclusion chromatography [79]	Applies a column packed with porous polymeric beads which separates the particles based on their size [79]	Allows precise separation of large and small molecules and application of various solutions. Compared to centrifugation methods, the structure of exosomes isolated by chromatography is not affected by shearing force [79]	Requires a long running time, which limits applications of chromatographical isolation for processing multiple biological samples [79]
Microfluidics [91]	Microscale isolation based on a variety of properties of exosomes like immunoaffinity, size, and density [91]	Energy efficient, portable, fast processing time, low cost, easy automation and integration [91]	Lack of standardization and large scale tests on clinical samples, lack of method validation, moderate to low sample capacity [91]
ExoQuick™ [91, 111]	Precipitates exosomes overnight through incubation [91, 111]	Fast and easy processing; additional equipment is not needed for isolation [91, 111]	Lack specificity toward exosomes; biological fluids are difficult to resuspend [91, 111]

**Table 1.** Advantages and disadvantages of isolation/purification methods.

debris, and large vesicles [79, 88]. In addition, these steps are used to precipitate exosomes [79, 88]. There are various protocols available for this isolation technique. First, cell culture is subjected to a low speed centrifugation using a Sorvall RT600 centrifuge with a swinging bucket rotor (Thermo Fisher Scientific). This is applied to remove cells and apoptotic debris [92, 93]. Next, a higher speed is used to administer and eliminate larger vesicles, whereas,

the remaining media is re-suspended in phosphate buffered saline. Lastly, a high speed of centrifugation using a SW41T1 swinging rotor in a Beckman Coulter (Brea, CA, USA) (Optima L-70 K ultracentrifuge) is performed to precipitate exosomes; and the exosome pellet is stored at  $-80^{\circ}\text{C}$  until further use [92].

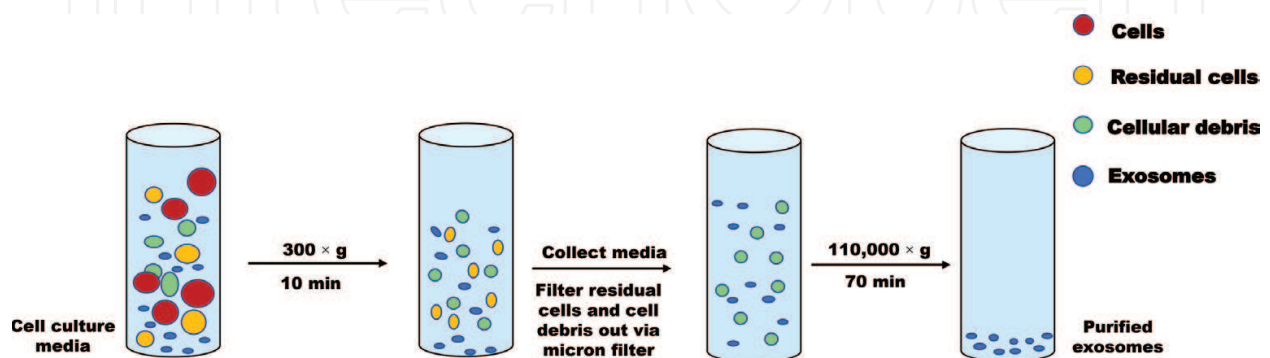
Filtration is a size-based technique that is often used in combination with ultracentrifugation, as depicted in **Figure 3**, for the isolation of exosomes in *in vitro* studies [92–94]. Depending on the size of vesicles, filtration is applied to separate exosomes from proteins and other particles [79]. Filtration membranes that have pore sizes of 0.22, 0.45, or 0.8  $\mu\text{m}$  can be used to collect EVs that are larger than 150 nm [79]. Although filtration is a quick isolation method, it faces challenges, such as contamination of isolated EVs, trapping of EVs in nano or micro pores, and co-purifying abundant proteins EVs isolation [91]. Because of these disadvantages, the maximal recovery of EVs for isolation must be optimized [91].

#### 4.2. Sucrose density gradient centrifugation

Sucrose density gradient centrifugation is a form of centrifugation that is used to measure the density of exosomes in a sucrose gradient [95]. Exosomes have floatation densities ranging from 1.08 to 1.22 g/ml on continuous sucrose gradients [91, 96, 97]. Vesicles that are purified from the Golgi float at 1.05 to 1.12 g/ml; and vesicles that are purified from the endoplasmic reticulum float at 1.18 to 1.25 g/ml [95]. Sucrose density gradient is formed by overlapping lower concentrations of sucrose on higher concentrations in a centrifuge tube. For instance, a sucrose gradient may contain layers ranging from 70% sucrose to 20% sucrose in 10% increments [91, 96, 97]. Since exosomes are generally spread among 3 to 5 segments of the sucrose gradient, it is recommended to perform this separation approximately 5 times the amount of exosomal proteins that is needed to detect exosomes.

#### 4.3. Size exclusion chromatography

Size exclusion chromatography (SEC) is used to separate macroparticles based on size, not molecular weight [79]. Currently, SEC is used to isolate exosomes that are present in urine [98] and blood [79, 99]. This method utilizes a column packed with porous polymeric beads



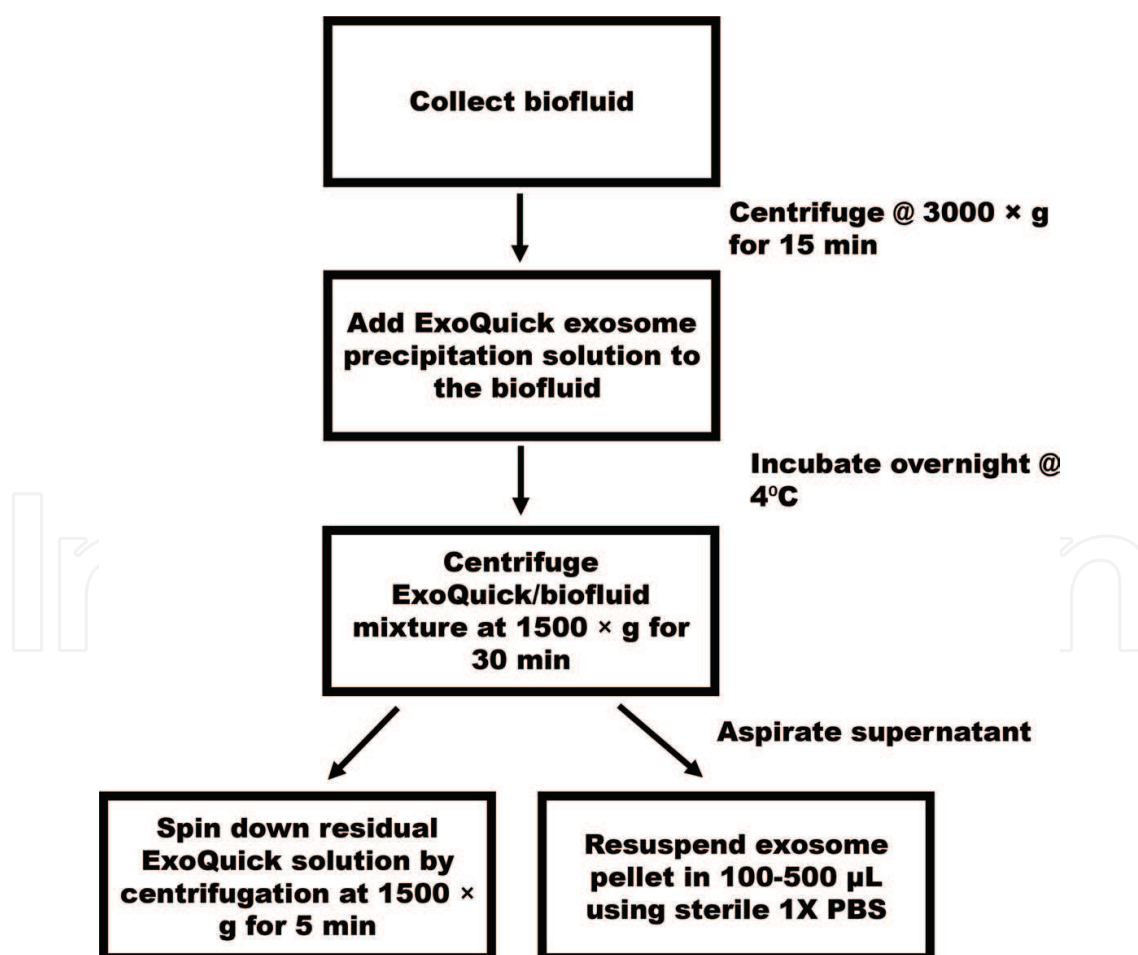
**Figure 3.** Schematic illustration of differential ultracentrifugation. Cell culture media is collected and centrifuged by means of low speed centrifugation followed by collection of media and filtration using a 0.22-micron filter. The media then undergoes ultracentrifugation pelleting the exosomes which are resuspended in buffer for further use.



that contains several pores and tunnels. In SEC, particles pass through the beads depending on their diameter. Particles that contain small hydrodynamic radii can pass through the pores, hence resulting in late elution [88]. However, particles that contain large hydrodynamic radii, are excluded from entering the pores [88, 100]. Correspondingly, SEC is used in combination with ultracentrifugation to isolate/purify exosomes [88, 101]. Rood et al. 2010 demonstrated that ultracentrifugation followed by SEC, significantly enriched urinary exosomes compared to exosomes that were obtained by ultrafiltration or ultracentrifugation alone [101, 102].

#### 4.4. Microfluidics

Microfluidics is the study and manipulation of fluids at the microscale level by means of frictional forces [91, 103]. Microfluidic devices bind specific EVs to antibody-coated surfaces [104, 105]. The EV sample is loaded on a pump that slowly pushes the fluid through the chip. Microfluidic-based technologies ensure that fluid pressure is converted to high shear forces more consistent and efficient than other technologies. By maintaining constant pressure, microfluidic homogenizers ensure that the samples receive the same treatment. As fluids are forced at controlled temperatures and constant pressures through the interaction chamber, particles



**Figure 4.** Standard ExoQuick™ protocol. Exosomes are collected by means of several centrifugation and precipitation steps.

experience extremely high shear forces. As a result of these forces, particle size is reduced and particle size distribution curves are constricted. Advantages of this technique include a reduction in processing times, energy consumption, volumes of sample, and material costs [106, 107].

#### 4.5. ExoQuick™

Commercial kits utilizing polyethylene glycol for isolation of exosomes are frequently used in research studies [108–110]. ExoQuick™ (System Biosciences, Mountain View, CA, USA) is the most commonly used kit [108]. This kit is quick and easy to perform, and additional equipment is not necessary for isolation. ExoQuick is a proprietary polymer that can be used to isolate exosomes for a variety of applications, including functional studies (i.e. cell-to-cell signaling), exosomal proteomics, biomarker studies, biology studies (i.e. tumorigenesis), exosomal miRNA profiling, and exosomal metabolomics/lipidomics [111–113]. With the ExoQuick, a mix is added to the samples and the EVs precipitate via incubation overnight. Recent studies have revealed that the highest yield of exosomes was obtained using a combination of ExoQuick with ultracentrifugation [91, 114, 115]. However, contamination of exosomal isolates with non-exosomal materials remains a concern for polymer-based isolation procedures. Furthermore, the polymer substance that is present in the isolate may affect the down-stream analysis [79]. A detailed protocol utilizing ExoQuick is depicted in **Figure 4**.

### 5. Exosome characterization methods

There are several common techniques that are used to determine the quantity, morphology, and size of exosomes following purification. Exosomes can be characterized using the following techniques: flow cytometry [116], electron microscopy (EM) [117], NanoSight tracking analysis (NTA) [92, 117, 118], Raman spectroscopy (RS) [119], Western blot (WB) [42, 92, 120], and/or ExoCarta database [117, 121].

Flow cytometry is one of the most commonly used techniques used to detect the origin, size, and morphology of circulating EVs [116, 121]. It is a high-throughput, multi-parametric technique that quickly analyzes and quantitates thousands of single cells or particles [121, 122]. In this method, a laser beam with a specific wavelength is directed through a stream of a sheath fluid that contains suspended particles [117]. Next, the emitted scatter and fluorescence is captured and measured by detectors [121]. Due to their small diameter ( $\leq 200$  nm), detecting, capturing, and examining exosomes is difficult to characterize via flow cytometry [123]. However, proteins that are located on the surface of exosomes can be stained with fluorochrome-conjugated antibodies [124].

EM is often used to characterize and visualize exosomes due their small size [117, 120]. EM uses a beam of electrons to generate an image of the EVs' sample [117]. Electron beams are passed through the sample [117]. The electrons are collected and magnified using special lenses [117]. Typical morphological characteristics of exosomes are spherical shaped and range approximately 30–100 nm [120]. When used in conjunction with immuno-labeling, the surface proteins of exosomes can be determined via electron microscopy [125].

NTA measures the concentration and size distribution of exosomes [92, 117, 126, 127]. An NTA device is composed of a laser light scattering microscope connected to a sensitive charge-coupled device camera, a complementary metal–oxide–semiconductor camera, a hydraulic pump, a measuring chamber, and an analytical software [117, 121]. The hydraulic pump injects particles into the measuring chamber at a fixed flow rate and exposes them to a narrow laser beam [117]. Next, the movement of the illuminated particles is recorded by the complementary metal-oxide-semiconductor [117]. The NTA software then identifies and tracks individual ECVs moving under Brownian motion and relates the movement to a particle size based on the Stokes-Einstein equation [128]:

$$(x, y)^2 = 2 k^B T / 3 r_h \pi \eta \quad (1)$$

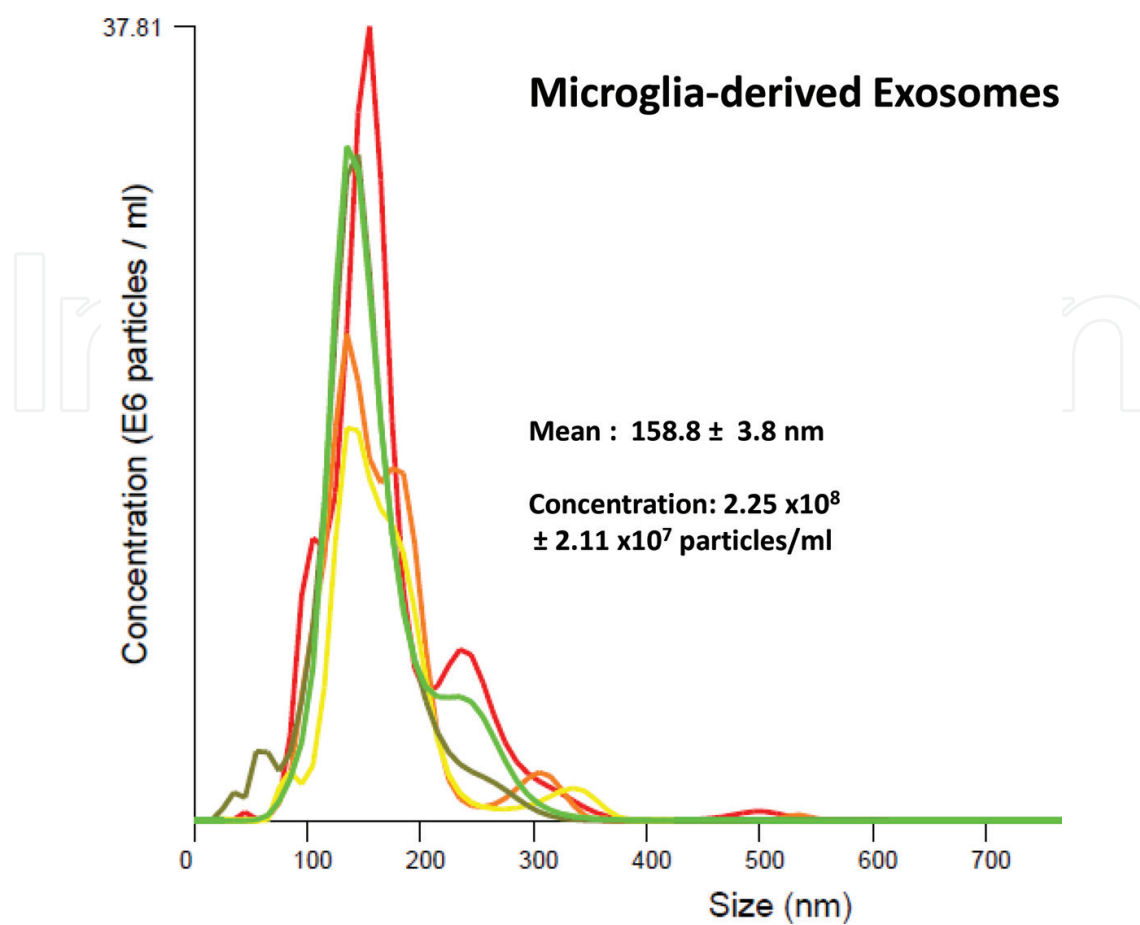
**Figure 5** depicts a graphic representation of the NTA.

RS is a quantitative technique that provides the chemical structure of exosomes based on the illumination of analyzed samples by laser light [129]. It is used to study rotational, vibrational, and other low-frequency transitions in a system [130]. Thus, it provides molecular fingerprints of the samples and enables monitoring of changes that occur in the molecular bond structures [117]. In this method, photons interact with other photons, molecular vibrations, and other excitations in the system. This interaction leads to a slight up or down shift of their energy. The shift in energy provides information about the vibrational transitions in the molecules [121, 130, 131]. Because of these measurements, the chemical composition of single EVs can be obtained [117, 131, 132].

WB is often used to show and confirm the presence of exosomal proteins and specific surface markers [133–135]. Specific surface markers include MHC I and MHC II, tetraspanins CD9, CD63, CD81, Hsp70 and Hsp90, etc. After EVs are isolated, they are lysed. Following lysis, the proteins are separated and analyzed [133]. Although WB is used to identify and confirm the presence of exosomal proteins, it cannot determine the presence of EVs alone. However, WB can be used to identify proteins in purified exosomal samples [120, 133].

Also, to help investigators validate and/or characterize their findings related to exosomes, researchers can use ExoCarta (<http://www.exocarta.org/>) [136]. ExoCarta is an online database that allows principal investigators the ability to identify and characterize exosomal cargos. The database contains detailed information about lipids, proteins, and RNA sequences that have been identified in specific exosomal preparations [77].

There are many other methods/techniques that are used to detect, identify, visualize, and characterize EVs. Additional techniques that have been used include: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) [137], Bradford assay [133], Enzyme-linked immunosorbent assay (ELISA) [133], dynamic light scattering (DLS) [117], mass spectrometry [137, 138], atomic force microscopy (AFM) [139], field-flow fractionation [140], and resistive pulse sensing [141]. Briefly, SDS PAGE, the Bradford assay, and ELISA are used to validate the presence of proteins. In this context, these assays could be used to confirm proteins on exosomes and/or proteins located within exosomes. Whereas, mass spectrometry, AFM, field-flow fractionation, and sensitive pulse sensing is to observe the molecular and physiochemical properties of EVs. These assays are often used to examine,

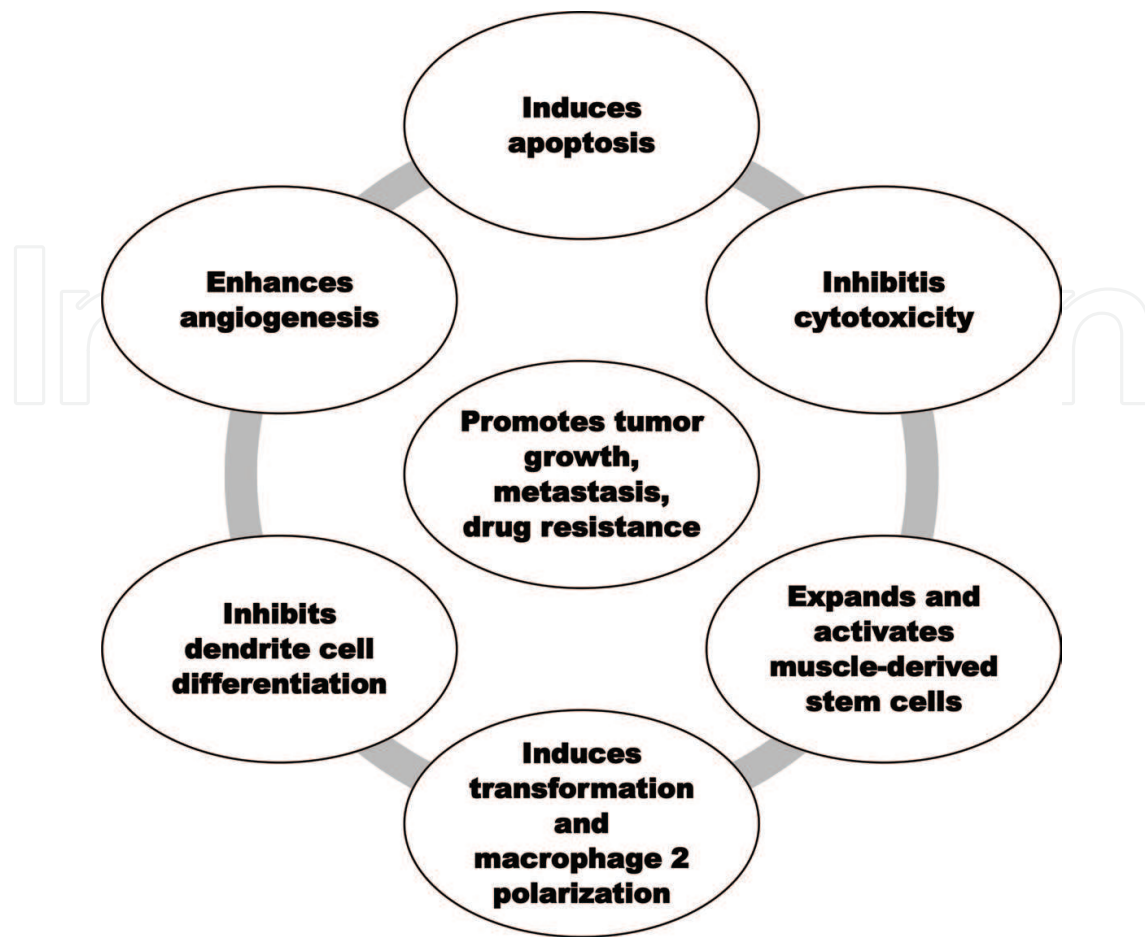


**Figure 5.** Representation of exosomes by NanoSight tracking analysis (NTA). Microglia-derived exosomes were generated as described in **Figure 3** and confirmed by NTA. In brief, we used the NanoSight LM10 (Malvern Instruments, Inc., Malvern, UK) and NTA v2.0 software to characterize mouse microglia-derived exosomes. All data were collected using five frames and in triplicate. Samples were diluted 1:1000 prior to tracking.

identify, and determine particle size and particle size distribution. Whenever applicable, statistical analyses should be performed to check results found from all methodologies. These analyses could include but are not limited to, Student T-test and analysis of variance (ANOVA).

## 6. Exosomes in cancer

Exosomes released from cancer cells may impact the cancer microenvironment significantly and alter the fate of proximal cells. These exosomes can mediate intracellular communication between other cancer cells, neighboring stromal cells, and immune cells [142–144]. In 2014, Boelens and team revealed that exosomes can be transferred from stromal cells to breast cancer cells [144, 145]. Due to this transfer, the antiviral retinoic acid-inducible gene 1 enzyme signaling can be activated to regulate the development of therapy-resistance tumor-initiating cells [144, 145]. Several studies have reported that cancer-derived exosomes play a major role in drug resistance, metastasis, angiogenesis, tumorigenesis, tumor growth, and tumor immune escape, as depicted in **Figure 6** [144, 146, 147].



**Figure 6.** Roles of exosomes in cancer. The multifaceted role of exosomes in carcinogenesis.

### 6.1. Drug resistance

Resistances to chemotherapy, radiation, or targeted therapies are significant challenges in the treatment of various cancers [148–150]. Recently, it has been demonstrated that exosomes aid in resistance through the transfer of lipids, proteins, mRNAs, and miRNAs [80, 151], which can influence the response to anticancer drugs [152]. Corcoran et al. [153] evaluated the enhancement of exosome secretion by Docetaxel-resistance in prostate cancer. They observed that this enhancement of exosome secretion is due to docetaxel efflux through exosomes [153]. Akao et al. [154] observed that the secretion of tumor-suppressors miRs-145 and miR-34 via exosomes increased 5-fluorouracil resistance in colon cancer cells.

### 6.2. Metastasis, angiogenesis, and tumorigenesis

It is believed that exosomes mediate signaling in cancer metastasis [155–158]. Exosomes can function as escape routes for miRNAs and proteins that serve as promoters of metastatic pathways. The uptake of exosomes by endothelial cells can stimulate angiogenesis [144, 159, 160]. Grange et al. [159] investigated the role of angiogenesis in renal cell-derived exosomes in lung cancer ascites. Exosomes that are stimulated by hypoxia and heparanase- an enzyme that acts at the cell surface and within the extracellular matrix to degrade heparan sulfate molecules, are associated with angiogenesis of breast cancer, which is the most significant



part of breast cancer tumorigenesis [145, 149]. Tumorigenesis is the process that occurs when normal healthy cells transform into cancerous cells. During this process, these cells can secrete exosomes [146, 147]. Several studies have reported that neoplastic transformation of adipose-derived stem cells was induced in response to prostate cancer-derived exosomes [146, 161]. Also, studies have reported that these exosomes deliver mRNA molecules and oncogenic proteins to recipient cells; which subsequently induced tumor formation [146, 162].

### **6.3. Tumor growth and tumor immune escape**

Tumor cells secrete many exosomes [163–166]. Supporting evidence has shown that exosomes released from tumors promote the formation of tumor blood vessels that support tumor growth and extension [144, 146, 149]. Glioblastoma multiforme cell-derived exosomes have been proposed to promote tumor growth by transporting RNA into recipient cells in the microenvironment [142, 143]. In 2011, Kogure and colleagues showed that hepatocellular carcinoma-derived exosomes can modify the transforming growth factor- $\beta$ -activated kinase 1 expression and associated signaling pathways to augment cell growth in recipient cells [144, 167].

Cancer cells utilize exosomes that contain proteins and nucleic acids to enact an immune escape [144]. It has been shown that they activate dendritic cells, thus priming the immune system to identify and kill cancer cells [168]. Remarkably, exosomes secreted by cancer cells have been proven to express tumor antigens, as well as immune suppressive molecules, such as Fas ligand and Programmed death-ligand 1 [169]. Taken together, these data suggest that cancer cells use exosomes to further advancement of its tumorigenesis.

## **7. Exosomes in genetic-related diseases**

Many types of cells (i.e. neurons, astrocytes, oligodendrocytes, glial) in the central nervous system secrete exosomes [32, 170–172]. Exosomes have been reported to aid in the spread of pathological proteins that are involved in neurodegenerative diseases, such as Alzheimer disease (AD) [170, 173], Parkinson's disease (PD) [171, 173, 174] prion diseases [32, 173] and Huntington's disease (HD) [32]. Current studies have shown that exosomes can spread pathological misfolded proteins, which leads to the onset and propagation of AD [170, 173]. AD is the most common form of dementia and characterized by amyloid plaques and neurofibrillary tangles [170, 173]. Accumulating evidence has demonstrated that exosomes play a controversial role in the pathogenesis of Alzheimer [170, 173, 175]. Yuyama et al. [176] observed the presence of exosome-associated amyloid- $\beta$  peptide in the cerebrospinal fluid of cynomolgus monkeys and amyloid precursor protein transgenic mice. They concluded that these findings could potentially contribute to AD pathogenesis [176].

Many studies have revealed that exosomes derived from the central nervous system occur in the cerebrospinal fluid and peripheral body fluids, and their contents are altered during disease, making them an appealing target for biomarker development in PD [171, 174]. PD is a disorder that occurs due to the loss of dopamine produced in the brain affecting movement of the body [172, 177]. Exosomes may aid in the spread of toxic  $\alpha$ -synuclein protein between

cells and induce apoptosis, which could potentially be proposed as a key mechanism underlying the spread of  $\alpha$ -synuclein aggregates in the brain and the acceleration of pathology in PD [171, 178]. Comparative studies have shown that the expression of the PD-associated protein  $\alpha$ -synuclein is targeted by miR-7 and miR-153 [179–181].

Prion diseases also known as transmissible spongiform encephalopathies are a group of infectious neurodegenerative disorders that affects animals and humans [172, 182]. These diseases are caused by abnormally shaped proteins called prions [177]. Exosome-mediated propagation in prion diseases was reported in 2004 by Fevrier et al. [183, 184]. They observed that the prion protein (PrP)-expressing cells could release normal PrP<sup>C</sup> and abnormal PrP<sup>Sc</sup> in association with exosomes [183, 184]. The first reported *in vivo* study related to prion disease pathogenesis was demonstrated 4 years later by Vella et al. 2008 [183, 185]. They revealed PrP<sup>C</sup> was associated with extracellular vesicles that were found in the CSF of sheep [183, 185].

HD is a hereditary neurodegenerative disorder that causes progressive degeneration of nerve cells in the brain due the aggregation of the mutant Huntingtin protein [186–188]. Lee et al. [186] investigated the therapeutic role of exosomes from adipose-derived stem cells by examining pathological phenotypes of a HD model *in vitro*. They confirmed that adipose stem cell-derived exosomes up-regulates the peroxisome proliferator-activated receptor gamma coactivator 1, phosphorylated cyclic AMP response element binding protein, and ameliorates abnormal apoptotic protein level in an *in vitro* HD model [186]. A year later, Soon-Tae Lee and colleagues developed an therapeutic exosome-based delivery method to treat HD using miR-124, one of the key miRNAs that is repressed in HD [189].

## 8. Exosomes in infectious diseases

### 8.1. Viruses

Exosomes derived from virus-infected cells have been shown to carry viral proteins, genetic regulatory elements, genomic RNA, mRNA, and miRNA [50, 190, 191]. Depending on the genetic material and proteins incorporated into them, EVs may play a vital role in viral infection, especially in retroviruses [192]. Retroviruses are enveloped RNA viruses that replicate through a DNA intermediate inserted in the host cell genome [193]. According to the Trojan hypothesis, it is believed that retroviruses exploit preexisting pathways for intracellular trafficking [192, 194]. Thus, the Trojan hypothesis states that retroviruses use the preexisting, nonviral exosome biogenesis pathway for the formation of infectious particles, and the preexisting, nonviral pathway of exosome uptake for a receptor-independent, enveloped-independent mode of infection [81, 194–196].

Among the retroviruses, HIV-1 is the most common studied virus [127, 197]. Exosomes isolated from patients with HIV infection or from HIV-1 infected cells incorporate the viral transactivating response element that is transcribed from the integrated provirus [50, 198]. This is believed to stimulate HIV-1 replication in recipient cells by downregulation of apoptosis [50, 197–199]. Madison et al. [200] showed that semen-derived exosomes inhibit HIV-1 replication

in various cell types. Years later, Madison and colleagues described detailed protocols for evaluating the function and physical properties of these semen-derived exosomes [200] for *in vitro* uptake and HIV-1 infection assays [201]. Recently, Sims et al. [92] have demonstrated the role of T cell immunoglobulin and mucin proteins (TIM) in exosome-dependent HIV-1 trafficking into human immune cells. Through viral infection assays, they demonstrated that exosomes derived from human lung carcinoma, human breast milk, human plasma, and mouse neural stem cells, increased HIV-1 entry into macrophages and T cells [92]. Furthermore, they demonstrated that HIV-1 and exosome interactions were potentially mediated through binding of TIM4 to the viral envelope [92]. In another study, Sims and colleagues demonstrated that exosomes can enhance HIV-1 entry into human monocytic and T cell lines through exosomal tetraspanin proteins CD9 and CD81 [127].

## 8.2. Bacteria-derived exosomes

Bacteria make and release membranous vesicles [202–204]. Gram-negative bacteria produce outer-membrane vesicles that originate from the blebbing of the outer membrane [202]. Also, they form vesicles that contain membrane components, nucleic acids, and proteins [202]. Many gram-negative bacteria that produce these vesicles are pathogenic and toxic to host cells [202, 205–207]. However, they can deliver antigens; and therefore, act as a potential vaccine candidate [202, 206, 207].

Gram-positive bacteria produce outer-membrane vesicles [202, 208]. Unlike gram-negative bacteria, these vesicles play a role in inter-species and intra-species communications [202, 209], in addition to potential inter-kingdom interaction with the host [202, 206, 210]. Most importantly, these vesicles provide an innovative approach for development of non-live vaccines [202]. These vaccines have been successfully used with children infected with *Neisseria meningitidis* in New Zealand [211].

## 8.3. Parasitic-derived exosomes

There is accumulating evidence that has reported the release of EVs in parasitic diseases, acting in parasite–parasite inter-communication and in parasite–host interactions [212–214]. EVs participate in the dissemination of the pathogen and play a vital role in host–pathogen interactions [212, 215]. Vesicles that are secreted by infected cells contain large amounts of pathogen molecules, which are sufficient to induce modifications in non-infected neighboring cells or act as antigen presenters for the immune system [215]. In 2013, Hassani and Olivier identified GP63 surface protease of *Leishmania mexicana* on exosomes [202, 216]. They observed that this protease could be transmitted to distant sites by enzymatic activity [202, 216].

# 9. Exosomes as diagnostic and therapeutic biomarkers

Exosomes have attracted enormous research interest because of their promising medical applications [217–219]. Exosomes may serve as diagnostic tools because they are carriers

of molecular markers of many diseases and as a prospective delivery system for various therapeutic agents [75, 220–222]. Supporting evidence suggests that exosomes are present in all bodily fluids and may be associated with disease pathogenesis [223–226] and may be involved in cellular protection [227, 228]. Mostly importantly, they contain various nucleic acids, lipids, and proteins. Due to the cargo of exosomes, exosomes are involved in several infectious diseases [75]. Because of their endocytic origin, exosomes carry specialized protein markers, such as hsps, tetraspanin, and Rab family proteins. Exosomal content is a fingerprint of the state (cancer versus quiescent) of the cell and the original cell type.

Exosomes can be used to diagnosis various diseases, such as cancer, AD, PD, HD, etc. [150, 173, 174, 186, 229]. Non-invasive diagnostics (using saliva and urine samples) or minimum invasive diagnostics (based on blood analysis) make exosomes very attractive alternatives to excision biopsies or traditional needle biopsies. There are advantages such as lower cost analysis, convenience and reduction in patient pain [229].

Exosomes can be exploited as potential carriers for therapeutics [230]. Many anti-inflammatory drugs (i.e. Doxorubicin) can be inserted into purified exosomes for *in vivo* and *in vitro* applications [231–235]. Sun et al. [236] investigated the anti-inflammatory activities of curcumin when encapsulated in exosomes. A year later, Zhuang and colleagues demonstrated that exosomes can be utilized to deliver anti-inflammatory drugs to the brain through a non-invasive intranasal route [232].

## 10. Summary

Secreted exosomes have important functions in the pathogenesis of various diseases. Several methods have been developed to isolate, purify, and characterize exosomes from biological fluids. However, isolation of exosomes can be problematic during the purification process due contaminants, such as protein aggregates, microvesicles, microbes, etc. Because of these contaminants, it is challenging to characterize exosomes accurately, and use them for experimental assays. Centrifugation techniques remain very common. However, other methods, such as filtration, sucrose density gradient centrifugation, SEC, microfluidics, and ExoQuick™ show promising results and can be effectively applied both in laboratory research and clinical medicine. It is most important to note that subsequent to exosome purification it is necessary to employ a combination of methods to confirm and characterize extracellular vesicles. The utilization of multimodality validations will allow researchers to obtain data that is qualitative, quantitative or both. Characterization of exosomes allows researchers to understand exosomal properties and function. Most importantly, characterization studies allow researchers to identify unique exosomal marker proteins to detect the presence of exosomes found in cell culture supernatants and biological fluids. The study of EV composition has shown that they can carry numerous cargos (i.e. lipids, proteins, and nucleic acids). These cargos can vary widely between cells and conditions. Their composition is cell type-dependent that can be altered by different environmental factors. The use of exosomes as therapeutic delivery vehicles covers a wide array of diseases, including but not limited to cancer, virus-induced diseases, genetically related diseases and parasitic diseases.

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## References

- [1] Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. *Current Opinion in Cell Biology*. 2014;**29**:116-125
- [2] Brintonn LT, Sloane H, Kester M, Kelly KA. Formation and role of exosomes in cancer. *Cellular and Molecular Life Sciences*. 2015;**72**(4):659-671
- [3] Harding C, Stahl P. Transferrin recycling in reticulocytes: pH and iron are important determinants of ligand binding and processing. *Biochemical and Biophysical Research Communications*. 1983;**11**(2):650-658
- [4] Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: Selective externalization of the receptor. *Cell*. 1983;**33**(3):967-78
- [5] De Broe ME, Wieme R, Logghe GN, Roels F. Spontaneous shedding of plasma membrane fragments by human cells in vivo and in vitro. *Clinica Chimica Acta*. 1977;**81**(3):237-245
- [6] De Broe ME, Borgers M, Wieme RJ. The separation and characterization of liver plasma membrane fragments circulating in the blood of patients with cholestasis. *Clinica Chimica Acta*. 1975;**59**(3):369-372
- [7] Brocklehurst D, Wilde C, Doar JW. The incidence and likely origins of serum particulate alkaline phosphatase and lipoprotein-X in liver disease. *Clinica Chimica Acta*. 1978;**88**(3):509-515
- [8] Pan BT, Teng K, Wu C, Adam M, Johnstone RM. Electron microscopic evidence for externalization of the transferin receptor in vesicular form in sheep reticulocytes. *The Journal of Cell Biology*. 1985;**101**(3):942-948



- [9] Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *The Journal of Biological Chemistry*. 1987;**262**(19):9412-9420
- [10] Joao AM, Alenquer M. Exosome biogenesis, regulation, and function in viral infection. *Viruses*. 2015;**7**(9):5066-5083
- [11] Harding CV, Heuser J, Stahl PD. Exosomes: Looking back three decades and into the future. *The Journal of Cell Biology*. 2013;**200**(4):367-371
- [12] Peter W. The nature and significance of platelet products in human plasma. *British Journal of Haematology*. 1967;**13**(3):269-288
- [13] Arraud N et al. Extracellular vesicles from blood plasma: Determination of their morphology, size, phenotype, and concentration. *Journal of Thrombosis and Haemostasis*. 2014;**12**(5):614-627
- [14] He C, Zheng S, Luo Y, Wang B. Exosome theranostics: Biology and translational medicine. *Theranostics*. 2018;**8**(1):237-255
- [15] Bobrie A, Colombo M, Raposo G, Théry C. Exosome secretion: Molecular mechanisms and roles in immune responses. *Traffic*. 2011;**12**(12):1659-1668
- [16] Raposo G, Stoorvogel W. Extracellular vesicles: Exosomes, microvesicles, and friends. *The Journal of Cell Biology*. 2013;**200**(4):373-383
- [17] Colombo M, Moita C, van Niel G, Kowal J, Vigneron J, Benaroch P, Manel N, Moita LF, Théry C, Raposo G. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *Journal of Cell Science*. 2013;**126**(24):5553-5565
- [18] Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature*. 2009;**458**(7237):445-452
- [19] Wollert T et al. The ESCRT machinery at a glance. *Journal of Cell Science*. 2009;**122**(13):2163-2166
- [20] Pocognoni CA, Berberian MV, Mayorga LS. ESCRT (Endosomal Sorting Complex Required for Transport) machinery is essential for acrosomal exocytosis in human sperm 1. *Biology of Reproduction*. 2015;**93**(5):124, 1-12-124, 1-12
- [21] Christ L et al. Cellular functions and molecular mechanisms of the ESCRT membrane-scission machinery. *Trends in Biochemical Sciences*. 2017;**42**(1):42-56
- [22] Kojima K, Amano Y, Yoshino K, Tanaka N, Sugamura K, Takeshita T. ESCRT-0 protein hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) is targeted to endosomes independently of signal-transducing adaptor molecule (STAM) and the complex formation with STAM promotes its endosomal dissociation. *Journal of Biological Chemistry*. 2015;**290**(13):8065-8066
- [23] Meister M, Bänfer S, Gärtner U, Koskimies J, Amaddii M, Jacob R, Tikkanen R. Regulation of cargo transfer between ESCRT-0 and ESCRT-I complexes by flotillin-1 during endosomal sorting of ubiquitinated cargo. *Oncogene*. 2017;**6**(6):e344

- [24] Wollert T et al. Membrane scission by the ESCRT-III complex. *Nature*. 2009;**458**:172-177
- [25] Saksena S et al. ESCRTing proteins in the endocytic pathway. *Trends in Biochemical Sciences*. 2007;**32**(12):561-573
- [26] Villarroya-Beltri C et al. Sorting it out: Regulation of exosome loading. *Seminars in Cancer Biology*. 2014;**28**:3-13
- [27] Landsberg MJ et al. Three-dimensional structure of AAA ATPase Vps4: Advancing structural insights into the mechanisms of endosomal sorting and enveloped virus budding. *Structure*. 2009;**17**(3):427-437
- [28] Allison B, Zhang H, Ratajczak MZ, Kakar SS. Exosomes: An overview of biogenesis, composition and role in ovarian cancer. *Journal of Ovarian Research*. 2014;**7**(14):1-11
- [29] Henderson MC, Azorsa DO. The genomic and proteomic content of cancer cell-derived exosomes. *Frontiers in Oncology*. 2012;**2**(38):1-9
- [30] Taylor DD, Gercel-Taylor C. Micro RNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecologic Oncology*. 2008;**110**(1):13-21
- [31] Hu G, Drescher KM, Chen XM. Exosomal miRNAs: Biological properties and therapeutic potential. *Frontiers in Genetics*. 2012;**3**(56):1-9
- [32] Chivet M, Hemming F, Pernet-Gallay K, Fraboulet S, Sadoul R. Emerging role of neuronal exosomes in the central nervous system. *Frontiers in Physiology*. 2012;**3**(145):1-6
- [33] Van Niel G, Mallegol J, Bevilacqua C, Candalh C, Brugière S, Tomaskovic-Crook E, Heath JK, Cerf-Bensussan N, Heyman M. Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. *Gut*. 2003;**52**(12):1690-1697
- [34] Kapsogeorgou EK, Abu-Helu R, Moutsopoulos HM, Manoussakis MN. Salivary gland epithelial cell exosomes: A source of autoantigenic ribonucleoproteins. *Arthritis and Rheumatism*. 2005;**52**(5):1517-1521
- [35] Théry C, Regnault A, Garin J, Wolfers J, Zitvogel L, Ricciardi-Castagnoli P, Raposo G, Amigorena S. Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. *The Journal of Cell Biology*. 1999;**147**(3):599-610
- [36] Zech D, Rana S, Büchler MW, Zöller M. Tumor-exosomes and leukocyte activation: An ambivalent crosstalk. *Cell Communication and Signaling*. 2012;**10**(1):1-17
- [37] Wang G, Dinkins M, He Q, Zhu G, Poirier C, Campbell A, Mayer-Proschel M, Bieberich E. Astrocytes secrete exosomes enriched with proapoptotic ceramide and prostate apoptosis response 4 (PAR-4): Potential mechanism of apoptosis induction in Alzheimer disease (AD). *The Journal of Biological Chemistry*. 2012;**287**(25):21384-21395
- [38] Zhan R, Leng X, Liu X, Wang X, Gong J, Yan L, Wang L, Wang Y, Wang X, Qian LJ. Heat shock protein 70 is secreted from endothelial cells by a non-classical pathway involving exosomes. *Biochemical and Biophysical Research Communications*. 2009;**387**(2):229-233

- [39] Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF, Kobayashi T, Salles JP, Perret B, Bonnerot C, Record M. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. *The Biochemical Journal*. 2004;**380**:161-171
- [40] Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Research*. 2010;**4**(3):214-122
- [41] Lässer C, ONS, Ekerljung L, Ekström K, Sjöstrand M, Lötval J. RNA-containing exosomes in human nasal secretions. *American Journal of Rhinology & Allergy*. 2011;**25**(2):89-93
- [42] Baranyai T, Herczeg K, Onódi Z, Voszka I, Módos K, Marton N, Nagy G, Mäger I, Wood MJ, El Andaloussi S, Pálkás Z, Kumar V, Nagy P, Kittel Á, Buzás EI, Ferdinandy P, Giricz Z. Isolation of exosomes from blood plasma: Qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods. *PLoS One*. 2015;**10**(12):1-13
- [43] Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One*. 2012;**7**(3):1-5
- [44] Keller S, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. *Journal of Translational Medicine*. 2011;**9**(86):1-9
- [45] Keller S, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S, Hager HD, Abdel-Bakky MS, Gutwein P, Altevogt P. CD24 is a marker of exosomes secreted into urine and amniotic fluid. *Kidney International*. 2007;**72**(9):1095-1102
- [46] Qin W, Tsukasaki Y, Dasgupta S, Mukhopadhyay N, Ikebe M, Sauter ER. Exosomes in human breast milk promote EMT. *Clinical Cancer Research*. 2016;**22**(17):4517-4524
- [47] Record M, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. *Biochemical Pharmacology*. 2011;**81**(10):1171-1182
- [48] Record M. Exosomal lipids in cell-cell communication. In: Z HG, editor. *Emerging Concepts of Tumor Exosome-Mediated Cell-Cell Communication*. New York, NY: Springer; 2013
- [49] Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nature Reviews. Immunology*. 2009;**9**(8):581-593
- [50] Chahar HS, Bao X, Casola A. Exosomes and their role in the life cycle and pathogenesis of RNA viruses. *Viruses*. 2015;**7**(3):3204-3225
- [51] Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiological. *Biochimica et Biophysica Acta*. 2014;**1841**(1):108-120
- [52] Sluijter JP, Verhage V, Deddens JC, van den Akker F, Doevendans PA. Microvesicles and exosomes for intracardiac communication. *Cardiovascular Research*. 2014;**102**(2):302-311
- [53] Ostrowski M, Carmo N, Krumeich S, Fanget I, Raposo G, Savina A, Moita CF, Schauer K, Hume AN, Freitas RP, Goud B, Benaroch P, Hacohen N, Fukuda M, Desnos C, Seabra

- MC, Darchen F, Amigorena S, Moita LF, Thery C. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nature Cell Biology*. 2010;**12**(1):19-30
- [54] Ponnambalam S, Baldwin S. Constitutive protein secretion from the trans-Golgi network to the plasma membrane (Review). 2003;**20**:129-139
- [55] Stenmark H, Stenmark H. Rab GTPases as coordinators of vesicle traffic. *Nature Reviews. Molecular Cell Biology*. 2009;**10**:513-525
- [56] Blanc L, Vidal M. New insights into the function of Rab GTPases in the context of exosomal secretion. *Small GTPases*. 2016;**9**(1-2):95-106
- [57] Pfeffer S. Rab GTPases: Specifying and deciphering organelle identity and function. *Trends in Cell Biology*. 2002;**11**:487-491
- [58] Hsu C, Morohashi Y, Yoshimura S, Manrique-Hoyos N, Jung SY, Lauterbach MA, Bakhti M, Grønborg M, Möbius W, Rhee JS, Barr FA, Simons M. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A–C. *Journal of Cell Biology*. 2010;**189**(2):223-232
- [59] Li G, Marlin MC, Rab GTPases. In: LG, editor. *Methods in Molecular Biology*. New York, NY: Humana Press; 2015
- [60] Hsu C, Morohashi Y, Yoshimura S, Manrique-Hoyos N, Jung S, Lauterbach MA, Bakhti M, Grønborg M, Möbius W, Rhee J, Barr FA, Simons M. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A. *The Journal of Cell Biology*. 2010;**189**(2):223-232
- [61] Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cellular and Molecular Life Sciences*. 2018;**75**(2):193-208
- [62] Savina A, Vidal M, Colombo MI. The exosome pathway in K562 cells is regulated by Rab11. *Journal of Cell Science*. 2002;**115**(12):2505-2515
- [63] Lindsay AJ, McCaffrey MW. Rabs of the Endosomal Recycling Pathway, in *Encyclopedia of Cell Biology*. Waltham: Academic Press; 2016. pp. 401-407
- [64] Lindsay AJ, MM. Rab11-FIP2 functions in transferrin recycling and associates with endosomal membranes via its COOH-terminal domain. *The Journal of Biological Chemistry*. 2002;**277**(30):27193-27199
- [65] Bouchet J et al. Rab11-FIP3 regulation of lck endosomal traffic controls TCR signal transduction. *The Journal of Immunology*. 2017;**198**(7):2967-2978
- [66] Ailawadi S, Wang X, Gu H, Fan GC. Pathologic function and therapeutic potential of exosomes in cardiovascular disease. *Biochimica et Biophysica Acta*. 2015;**1852**(1):1-11
- [67] Yu X, Harris S, Levine AJ. The regulation of exosome secretion: A novel function of the p53 protein. *Cancer Research*. 2006;**66**(9):4795-4801
- [68] Keller S, Sanderson M, Stoeck A, Altevogt P, Exosomes: From biogenesis and secretion to biological function. *Immunology Letters*. 2006;**107**(2):102-108



- [69] Gross JC et al. Active Wnt proteins are secreted on exosomes. *Nature Cell Biology*. 2012;**14**:1036
- [70] Lespagnol A, Duflaut D, Beekman C, Blanc L, Fiucci G, Marine JC, Vidal M, Amson R, Telerman A. Exosome secretion, including the DNA damage-induced p53-dependent secretory pathway, is severely compromised in TSAP6/Steap3-null mice. *Cell Death and Differentiation*. 2008;**15**(11):1723-1733
- [71] King HW, Michael M, Gleadle JM. Hypoxic enhancement of exosome release by breast cancer cells. *BMC Cancer*. 2012;**212**(421):1-10
- [72] Harris AL. Hypoxia—A key regulatory factor in tumour growth. *Nature Reviews. Cancer*. 2002;**2**(1):38-47
- [73] Hooper C et al. Wnt3a induces exosome secretion from primary cultured rat microglia. 2012;**13**:144
- [74] Boriachek K et al. Biological functions and current advances in isolation and detection strategies for exosome nanovesicles. *Small Journal*. 2017;**14**:1702153
- [75] Lin J et al. Exosomes: Novel biomarkers for clinical diagnosis. *The Scientific World Journal*. 2015;**2015**:8
- [76] Alipoor SD, Mortaz E, Garssen J, Movassaghi M, Mirsaeidi M, Adcock IM. Exosomes and exosomal miRNA in respiratory diseases. *Mediators of Inflammation*. 2016;**2016**:1-11
- [77] Mathivanan S et al. ExoCarta 2012: Database of exosomal proteins, RNA and lipids. *Nucleic Acids Research*. 2012;**40**:D1241-D1244
- [78] Clotilde T, Zitvogel L, Amigorena S. Exosomes: Composition, biogenesis and function. *Nature Reviews Immunology*. 2002;**2**:569-579
- [79] Yakimchuk K. Exosomes: Isolation and characterization methods and specific markers. *Labome. Mater Methods*. 2015;**5**(1450):ref79retrieved from <https://labome.com/method>
- [80] Kalluri R. The biology and function of exosomes in cancer. *The Journal of Clinical Investigation*. 2016;**126**(4):1208-1215
- [81] Booth AM et al. Exosomes and HIV Gag bud from endosome-like domains of the T cell plasma membrane. *The Journal of Cell Biology*. 2006;**172**(6):923-935
- [82] Bang C, Thum T. Exosomes: New players in cell–cell communication. *The International Journal of Biochemistry & Cell Biology*. 2012;**44**(11):2060-2064
- [83] Teresa J et al. Mechanisms of RNA loading into exosomes. *FEBS Letters*. 2015;**589**(13):1391-1398
- [84] Zhang J et al. Exosome and exosomal microRNA: Trafficking, sorting, and function. *Genomics, Proteomics & Bioinformatics*. 2015;**13**(1):17-24
- [85] Behbahani GD et al. The role of exosomes contents on genetic and epigenetic alterations of recipient cancer cells. *Iranian Journal of Basic Medical Sciences*. 2016;**19**(10):1031-1039



- [86] Valadi H et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*. 2007;**9**:654
- [87] Szatanek R, Baran J, Siedlar M, Baj-Krzyworzeka M. Isolation of extracellular vesicles: Determining the correct approach (Review). *International Journal of Molecular Medicine*. 2015;**36**(1):11-17
- [88] Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. *Theranostics*. 2017;**7**(3):789-804
- [89] Khatun Z, Bhat A, Sharma S, Sharma A. Elucidating diversity of exosomes: Biophysical and molecular characterization methods. *Nanomedicine*. 2016;**11**(17):2359-2377
- [90] Willis GR, Kourembanas S, Mitsialis SA. Toward exosome-based therapeutics: Isolation, heterogeneity, and fit-for-purpose potency. *Frontiers in Cardiovascular Medicine*. 2017;**4**(63):1-13
- [91] Momen-Heravi F, Balaj L, Alian S, Mantel PY, Halleck AE, Trachtenberg AJ, Soria CE, Oquin S, Bonebreak CM, Saracoglu E, Skog J, Kuo WP, Current methods for the isolation of extracellular vesicles. *Biological Chemistry*. 2013;**394**(10):1253-1262
- [92] Sims B, Farrow AL, Williams SD, Bansal A, Krendelchtchikov A, Gu L, Matthews QL. Role of TIM-4 in exosome-dependent entry of HIV-1 into human immune cells. *International Journal of Nanomedicine*. 2017;**12**:4823-4833
- [93] Sims B, Gu L, Krendelchtchikov A, Matthews QL. Neural stem cell-derived exosomes mediate viral entry. *International Journal of Nanomedicine*. 2014;**9**:4893-4897
- [94] Nordin J, Lee Y, Vader P, Mäger I, Johansson HJ, Heusermann W, Wiklander OP, Hällbrink M, Seow Y, Bultema JJ, Gilthorpe J, Davies T1, Fairchild PJ, Gabrielsson S, Meisner-Kober NC, Lehtiö J, Smith CI, Wood MJ, El Andaloussi S. Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties. *Nanomedicine*. 2015;**11**(4):879-883
- [95] Théry C et al. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Current Protocols in Cell Biology*. 2006;**30**(1):3.22.1-3.22.29
- [96] Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. *The Journal of Experimental Medicine*. 1996;**183**(3):1161-1172
- [97] Cantin R, Diou J, Bélanger D, Tremblay AM, Gilbert C. Discrimination between exosomes and HIV-1: Purification of both vesicles from cell-free supernatants. *Journal of Immunological Methods*. 2008;**338**(1-2):21-30
- [98] Lozano-Ramos I, Bancu I, Oliveira-Tercero A, et al. Size-exclusion chromatography-based enrichment of extracellular vesicles from urine samples. *Journal of Extracellular Vesicles*. 2015;**4**:1-11
- [99] Taylor DD, LK, Gerçel-Taylor C. Shed membrane fragment-associated markers for endometrial and ovarian cancers. *Gynecologic Oncology*. 2002;**84**(3):443-448

- [100] Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. *PLOS One*. 2014;**9**(2):1-8
- [101] Rood IM, DJ, Merchant ML, Tamboer WP, Wilkey DW, Wetzels JF, et al, Comparison of three methods for isolation of urinary microvesicles to identify biomarkers of nephrotic syndrome. *Kidney International*. 2010;**78**:810-816
- [102] Musante L, Tataruch DE, Holthofer H, Use and isolation of urinary exosomes as biomarkers for diabetic nephropathy. *Frontiers in Endocrinology*. 2014;**5**(149).1-12
- [103] Velve-Casquillas G, Le Berre M, Piel M, Tran PT. Microfluidic tools for cell biological research. *Nano Today*. 2010;**5**(1):28-47
- [104] Huang-Doran I, Zhang C-Y, Vidal-Puig A. Extracellular vesicles: Novel mediators of cell communication in metabolic disease. *Trends in Endocrinology & Metabolism*. 2017;**28**(1):3-18
- [105] Gholizadeh S et al. Microfluidic approaches for isolation, detection, and characterization of extracellular vesicles: Current status and future directions. *Biosensors and Bioelectronics*. 2017;**91**:588-605
- [106] Halldorsson S et al. Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. *Biosensors and Bioelectronics*. 2015;**63**:218-231
- [107] Streets AM, Huang Y. Chip in a lab: Microfluidics for next generation life science research. *Biomicrofluidics*. 2013;**7**(1):011302
- [108] Caradec J et al. Reproducibility and efficiency of serum-derived exosome. Extraction methods. *Clinical Biochemistry*. 2014;**47**:1286-1292
- [109] Rider MA, Hurwitz SN, Meckes D. ExtraPEG: A polyethylene glycol-based method for enrichment of extracellular vesicles. *Scientific Reports*. 2016;**6**:23978
- [110] Kenigsberg S et al. Protocol for exosome isolation from small volume of ovarian follicular fluid: Evaluation of ultracentrifugation and commercial kits. In: Kuo WP, Jia S, editors. *Extracellular Vesicles: Methods and Protocols*. New York, NY: Springer; 2017. pp. 321-341
- [111] System Biosciences. ExoQuick. Retrieved from <https://www.systembio.com/>
- [112] Barreiro K, Holthofer H. Urinary extracellular vesicles. A promising shortcut to novel biomarker discoveries. *Cell and Tissue Research*. 2017;**369**(1):217-227
- [113] Verma M et al. Extracellular vesicles: Potential applications in cancer diagnosis, prognosis, and epidemiology. *BMC Clinical Pathology*. 2015;**15**(1):6
- [114] Yamada T, Inoshima Y, Matsuda T, Ishiguro N. Comparison of methods for isolating exosomes from bovine milk. *The Journal of Veterinary Medical Science*. 2012;**74**(11): 1523-1525

- [115] Shin H et al. High-yield isolation of extracellular vesicles using aqueous two-phase system. *Scientific Reports*. 2015;**5**:13103
- [116] Kim HK et al. Optimized flow cytometric assay for the measurement of platelet microparticles in plasma: Pre-analytic and analytic considerations. *Blood Coagulation & Fibrinolysis*. 2002;**13**(5):393-397
- [117] Szatanek R et al. The methods of choice for extracellular vesicles (EVs) characterization. *International Journal of Molecular Sciences*. 2017;**18**(6):1-18
- [118] Mehdiani A et al. An innovative method for exosome quantification and size measurement. *Journal of Visualized Experiments*. 2015;**95**(e50974):1-9
- [119] Tirinato L et al. SERS analysis on exosomes using super-hydrophobic surfaces. *Micro-electronic Engineering*. 2012;**97**:337-340
- [120] Lasser C, Eldh M, Lotvall J. Isolation and characterization of RNA-containing exosomes. *Journal of Visualized Experiments*. 2012;**59**:e3037
- [121] Momen-Heravi F et al. Alternative methods for characterization of extracellular vesicles. *Frontiers in Physiology*. 2012;**3**:354
- [122] van der Pol E et al. Single vs. swarm detection of microparticles and exosomes by flow cytometry. *Journal of Thrombosis and Haemostasis*. 2012;**10**(5):919-930
- [123] Welsh JA et al. Extracellular vesicle flow cytometry analysis and standardization. *Frontiers in Cell and Development Biology*. 2017;**5**:78
- [124] Morales-Kastresana A, Jones JC. Flow cytometric analysis of extracellular vesicles. *Methods in Molecular Biology*. 2017;**1545**:215-225
- [125] Witwer KW et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *Journal of Extracellular Vesicles*. 2013;**2**:1-25
- [126] Dragovic RA et al. Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. *Nanomedicine*. 2011;**7**(6):780-788
- [127] Sims B et al. Tetraspanin blockage reduces exosome-mediated HIV-1 entry. *Archives of Virology*. 2018;**163**(6):1683-1689
- [128] Filipe V, Hawe A, Jiskoot W. Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. *Pharmaceutical Research*. 2010;**27**(5):796-810
- [129] Smith ZJ et al. Single exosome study reveals subpopulations distributed among cell lines with variability related to membrane content. *Journal of Extracellular Vesicles*. 2015;**4**:28533
- [130] Puppels GJ et al. Studying single living cells and chromosomes by confocal Raman microspectroscopy. *Nature*. 1990;**347**(6290):301-303

- [131] van der Pol E et al. Optical and non-optical methods for detection and characterization of microparticles and exosomes. *Journal of Thrombosis and Haemostasis*. 2010; **8**(12):2596-2607
- [132] Tatischeff I et al. Fast characterisation of cell-derived extracellular vesicles by nanoparticles tracking analysis, cryo-electron microscopy, and Raman tweezers microspectroscopy. *Journal of Extracellular Vesicles*. 2012; **1**:1-11
- [133] Sunkara V, Woo HK, Cho YK. Emerging techniques in the isolation and characterization of extracellular vesicles and their roles in cancer diagnostics and prognostics. *Analyst*. 2016; **141**(2):371-381
- [134] Rho J et al. Magnetic nanosensor for detection and profiling of erythrocyte-derived microvesicles. *ACS Nano*. 2013; **7**(12):11227-11233
- [135] He M et al. Integrated immunoisolation and protein analysis of circulating exosomes using microfluidic technology. *Lab on a Chip*. 2014; **14**(19):3773-3780
- [136] Simpson RJ, Kalra H, Mathivanan S. ExoCarta as a resource for exosomal research. *Journal of Extracellular Vesicles*. 2012; **1**:1-6
- [137] Wubbolts R et al. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. *The Journal of Biological Chemistry*. 2003; **278**(13):10963-10972
- [138] Abramowicz A, Widlak P, Pietrowska M. Proteomic analysis of exosomal cargo: The challenge of high purity vesicle isolation. *Molecular BioSystems*. 2016; **12**(5):1407-1419
- [139] Sharma S et al. Quantitative nanostructural and single-molecule force spectroscopy biomolecular analysis of human-saliva-derived exosomes. *Langmuir*. 2011; **27**(23):14394-14400
- [140] Schachermeyer S, Ashby J, Zhong W. Advances in field-flow fractionation for the analysis of biomolecules: Instrument design and hyphenation. *Analytical and Bioanalytical Chemistry*. 2012; **404**(4):1151-1158
- [141] Graham MD. The Coulter principle: Imaginary origins. *Cytometry. Part A*. 2013; **83**(12):1057-1061
- [142] Ung TH et al. Exosome proteomics reveals transcriptional regulator proteins with potential to mediate downstream pathways. *Cancer Science*. 2014; **105**(11):1384-1392
- [143] Skog J et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature Cell Biology*. 2008; **10**(12):1470-1476
- [144] Guo W et al. Exosomes: New players in cancer (Review). *Oncology Reports*. 2017; **38**(2):665-675
- [145] Boelens MC et al. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. *Cell*. 2014; **159**(3):499-513

- [146] Oves M et al. Exosomes: A paradigm in drug development against cancer and infectious diseases. *Journal of Nanomaterials*. 2018;**2018**:17
- [147] Zhang X et al. Exosomes in cancer: Small particle, big player. *Journal of Hematology & Oncology*. 2015;**8**:83
- [148] Andre Mdo R, Pedro A, Lyden D. Cancer exosomes as mediators of drug resistance. *Methods in Molecular Biology*. 2016;**1395**:229-239
- [149] Yu DD et al. Exosomes in development, metastasis and drug resistance of breast cancer. *Cancer Science*. 2015;**106**(8):959-964
- [150] Bach DH et al. The role of exosomes and miRNAs in drug-resistance of cancer cells. *International Journal of Cancer*. 2017;**141**(2):220-230
- [151] Wang X et al. Exosomes play an important role in the process of psoralen reverse multidrug resistance of breast cancer. *Journal of Experimental & Clinical Cancer Research*. 2016;**35**(1):186
- [152] Ohno S-i, Ishikawa A, Kuroda M. Roles of exosomes and microvesicles in disease pathogenesis. *Advanced Drug Delivery Reviews*. 2013;**65**(3):398-401
- [153] Corcoran C, RS, O'Brien K, O'Neill A, Prencipe M, Sheikh R, et al. Docetaxel-resistance in prostate cancer: Evaluating associated phenotypic changes and potential for resistance transfer via exosomes. *PLoS One*. 2012;**7**(12):e50999
- [154] Akao Y et al. Extracellular disposal of tumor-suppressor miRs-145 and -34a via microvesicles and 5-FU resistance of human colon cancer cells. *International Journal of Molecular Sciences*. 2014;**15**(1):1392-1401
- [155] Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: A comprehensive review. *Cancer Metastasis Reviews*. 2013;**32**(3-4):623-642
- [156] Weidle UH et al. The multiple roles of exosomes in metastasis. *Cancer Genomics Proteomics*. 2017;**14**(1):1-15
- [157] Zhang C et al. Exosome: Function and role in cancer metastasis and drug resistance. *Technology in Cancer Research & Treatment*. 2018;**17**:1533033818763450
- [158] Becker A et al. Extracellular vesicles in cancer: Cell-to-cell mediators of metastasis. *Cancer Cell*. 2016;**30**(6):836-848
- [159] Grange C et al. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Research*. 2011;**71**(15):5346-5356
- [160] Polet F, Feron O. Endothelial cell metabolism and tumour angiogenesis: Glucose and glutamine as essential fuels and lactate as the driving force. *Journal of Internal Medicine*. 2013;**273**(2):156-165



- [161] Abd Elmageed ZY et al. Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. *Stem Cells*. 2014;**32**(4):983-997
- [162] Kahlert C, Kalluri R. Exosomes in tumor microenvironment influence cancer progression and metastasis. *Journal of Molecular Medicine (Berl)*. 2013;**91**(4):431-437
- [163] Kalra H, D.G., Mathivanan S, Focus on extracellular vesicles: Introducing the next small big thing. *International Journal of Molecular Sciences*. 2016;**17**(2):1-30
- [164] Logozzi M et al. High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients. *PLoS One*. 2009;**4**(4):e5219
- [165] Whiteside TL. Tumor-derived exosomes and their role in cancer progression. *Advances in Clinical Chemistry*. 2016;**74**:103-141
- [166] Atay S, Godwin AK. Tumor-derived exosomes: A message delivery system for tumor progression. *Communicative & Integrative Biology*. 2014;**7**(1):e28231
- [167] Kogure T et al. Intercellular nanovesicle-mediated microRNA transfer: A mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology*. 2011;**54**(4):1237-1248
- [168] Tan A, De La Pena H, Seifalian AM. The application of exosomes as a nanoscale cancer vaccine. *International Journal of Nanomedicine*. 2010;**5**:889-900
- [169] Ichim TE et al. Exosomes as a tumor immune escape mechanism: Possible therapeutic implications. *Journal of Translational Medicine*. 2008;**6**:37
- [170] Kawikova I, Askenase PW. Diagnostic and therapeutic potentials of exosomes in CNS diseases. *Brain Research*. 2015;**1617**:63-71
- [171] Rajendran L et al. Emerging roles of extracellular vesicles in the nervous system. *The Journal of Neuroscience*. 2014;**34**(46):15482-15489
- [172] Kanninen KM et al. Exosomes as new diagnostic tools in CNS diseases. *Biochimica et Biophysica Acta*. 2016;**1862**(3):403-410
- [173] Xiao T et al. The role of exosomes in the pathogenesis of Alzheimer' disease. *Translational Neurodegeneration*. 2017;**6**:3
- [174] Wu X, Zheng T, Zhang B. Exosomes in Parkinson's disease. *Neuroscience Bulletin*. 2017;**33**(3):331-338
- [175] Vella L, Hill A, Cheng L. Focus on extracellular vesicles: Exosomes and their role in protein trafficking and biomarker potential in Alzheimer's and Parkinson's disease. *International Journal of Molecular Sciences*. 2016;**17**:173
- [176] Yuyama K et al. A potential function for neuronal exosomes: Sequestering intracerebral amyloid- $\beta$  peptide. *FEBS Letters*. 2014;**589**
- [177] Geschwind MD. Prion diseases. *Continuum (Minneap. Minn.)*. 2015;**21**(6 Neuroinfectious Disease):1612-1638

- [178] Baksi S, Singh N. Alpha-synuclein impairs ferritinophagy in the retinal pigment epithelium: Implications for retinal iron dyshomeostasis in Parkinson's disease. *Scientific Reports*. 2017;**7**(1):12843
- [179] Karnati HK et al. miRNAs: Key players in neurodegenerative disorders and epilepsy. *Journal of Alzheimer's Disease*. 2015;**48**(3):563-580
- [180] Wang G et al. Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein. *American Journal of Human Genetics*. 2008;**82**(2):283-289
- [181] Doxakis E. Post-transcriptional regulation of alpha-synuclein expression by mir-7 and mir-153. *The Journal of Biological Chemistry*. 2010;**285**(17):12726-12734
- [182] Burchell JT, Panegyres PK. Prion diseases: Immunotargets and therapy. *ImmunoTargets and Therapy*. 2016;**5**:57-68
- [183] Soria F et al. Exosomes, an unmasked culprit in neurodegenerative diseases. *Frontiers in Neuroscience*. 2017;**11**:1-12
- [184] Fevrier B et al. Cells release prions in association with exosomes. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**(26):9683-9688
- [185] Vella LJ et al. Enrichment of prion protein in exosomes derived from ovine cerebral spinal fluid. *Veterinary Immunology and Immunopathology*. 2008;**124**(3-4):385-393
- [186] Lee M et al. Exosomes from adipose-derived stem cells ameliorate Huntington's disease phenotypes in an in vitro model. *European Journal of Neuroscience*. 2016;**44**:2114-2119
- [187] Hong Y et al. Mutant Huntingtin inhibits  $\alpha$ B-crystallin expression and impairs exosome secretion from astrocytes. *The Journal of Neuroscience*. 2017;**37**(39):9550-9563
- [188] Sari Y. Huntington's disease: From mutant Huntingtin protein to neurotrophic factor therapy. *International Journal of Biomedical Science*. 2011;**7**(2):89-100
- [189] Lee S-T et al. Exosome-based delivery of miR-124 in a Huntington's disease model. *Journal of Movement Disorders*. 2017;**10**:45-52
- [190] Anderson MR, Kashanchi F, Jacobson S. Exosomes in viral disease. *Neurotherapeutics*. 2016;**13**(3):535-546
- [191] Raab-Traub N, Dittmer DP. Viral effects on the content and function of extracellular vesicles. *Nature Reviews. Microbiology*. 2017;**15**(9):559-572
- [192] Nolte-'t Hoen E et al. Extracellular vesicles and viruses: Are they close relatives? *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(33):9155-9161
- [193] Coffin JM, Hughes SH, Varmus HE, editors. *Retroviruses*. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997
- [194] Gould SJ, Booth AM, Hildreth JE. The Trojan exosome hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;**100**(19):10592-10597

- [195] Hildreth JEK. HIV as trojan exosome: Immunological paradox explained? *Frontiers in Immunology*. 2017;**8**:1715
- [196] Nguyen DG et al. Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. *The Journal of Biological Chemistry*. 2003;**278**(52):52347-52354
- [197] Ellwanger JH, Veit TD, Chies JAB. Exosomes in HIV infection: A review and critical look. *Infection, Genetics and Evolution*. 2017;**53**:146-154
- [198] Madison MN, Okeoma CM. Exosomes: Implications in HIV-1 pathogenesis. *Viruses*. 2015;**7**(7):4093-4118
- [199] Narayanan A et al. Exosomes derived from HIV-1-infected cells contain trans-activation response element RNA. *The Journal of Biological Chemistry*. 2013;**288**(27):20014-20033
- [200] Madison MN, Roller RJ, Okeoma CM. Human semen contains exosomes with potent anti-HIV-1 activity. *Retrovirology*. 2014;**11**:102
- [201] Madison MN, Welch JL, Okeoma CM. Isolation of exosomes from semen for in vitro uptake and HIV-1 infection assays. *Bio-protocol*. 2017;**7**(7):1-27
- [202] Schwab A et al. Extracellular vesicles from infected cells: Potential for direct pathogenesis. *Frontiers in Microbiology*. 2015;**6**:1132
- [203] Deatherage BL, Cookson BT. Membrane vesicle release in bacteria, eukaryotes, and archaea: A conserved yet underappreciated aspect of microbial life. *Infection and Immunity*. 2012;**80**(6):1948-1957
- [204] Rodrigues M et al. Role of extracellular vesicles in viral and bacterial infections: Pathogenesis, diagnostics, and therapeutics. *Theranostics*. 2018;**8**(10):2709-2721
- [205] Kadurugamuwa JL, Beveridge TJ. Membrane vesicles derived from *Pseudomonas aeruginosa* and *Shigella flexneri* can be integrated into the surfaces of other gram-negative bacteria. *Microbiology*. 1999;**145**(Pt 8):2051-2060
- [206] Pierson T et al. Proteomic characterization and functional analysis of outer membrane vesicles of *Francisella novicida* suggests possible role in virulence and use as a vaccine. *Journal of Proteome Research*. 2011;**10**(3):954-967
- [207] Nieves W et al. A *Burkholderia pseudomallei* outer membrane vesicle vaccine provides protection against lethal sepsis. *Clinical and Vaccine Immunology*. 2014;**21**(5):747-754
- [208] Brown L et al. Extracellular vesicles produced by the Gram-positive bacterium *Bacillus subtilis* are disrupted by the lipopeptide surfactin. *Molecular Microbiology*. 2014;**93**(1):183-198
- [209] Berleman J, Auer M. The role of bacterial outer membrane vesicles for intra- and inter-species delivery. *Environmental Microbiology*. 2013;**15**(2):347-354

- [210] Kulp A, Kuehn MJ. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. *Annual Review of Microbiology*. 2010;**64**:163-184
- [211] Wong SH et al. Immunogenicity and tolerability in infants of a New Zealand epidemic strain meningococcal B outer membrane vesicle vaccine. *The Pediatric Infectious Disease Journal*. 2009;**28**(5):385-390
- [212] Marcilla A et al. Extracellular vesicles in parasitic diseases. *Journal of Extracellular Vesicles*. 2014;**3**:25040
- [213] Coakley G, Maizels RM, Buck AH. Exosomes and other extracellular vesicles: The new communicators in parasite infections. *Trends in Parasitology*. 2015;**31**(10):477-489
- [214] Pontes L et al. Biomarkers in exosomes in cattle diseases. Research Gate. 2018. Conference article
- [215] Atayde VD et al. Exosome secretion by the parasitic protozoan leishmania within the sand fly midgut. *Cell Reports*. 2015;**13**(5):957-967
- [216] Hassani K, Olivier M. Immunomodulatory impact of leishmania-induced macrophage exosomes: A comparative proteomic and functional analysis. *PLoS Neglected Tropical Diseases*. 2013;**7**(5):e2185
- [217] Dorayappan KDP et al. The biological significance and clinical applications of exosomes in ovarian cancer. *Gynecologic Oncology*. 2016;**142**(1):199-205
- [218] Gyorgy B et al. Therapeutic applications of extracellular vesicles: Clinical promise and open questions. *Annual Review of Pharmacology and Toxicology*. 2015;**55**:439-464
- [219] Santangelo L et al. Functional roles and therapeutic applications of exosomes in *Hepatocellular Carcinoma*. *BioMed Research International*. 2017;**2017**:8
- [220] De Toro J, Waldner C, Mongini C. Emerging roles of exosomes in normal and pathological conditions: New insights for diagnosis and therapeutic applications. *Frontiers in Immunology*. 2015;**6**(203):1-12
- [221] Lasser C. Exosomes in diagnostic and therapeutic applications: Biomarker, vaccine and RNA interference delivery vehicle. *Expert Opinion on Biological Therapy*. 2015;**15**(1):103-117
- [222] Dommelen S et al. Microvesicles and exosomes: Opportunities for cell-derived membrane vesicles in drug delivery;**161**, 2011:635-644
- [223] Cappello F et al. Exosome levels in human body fluids: A tumor marker by themselves? *European Journal of Pharmaceutical Sciences*. 2017;**96**:93-98
- [224] Barile L, Vassalli G. Exosomes: Therapy delivery tools and biomarkers of diseases. *Pharmacology & Therapeutics*. 2017;**174**:63-78
- [225] Delenclos M et al. Investigation of endocytic pathways for the internalization of exosome-associated oligomeric alpha-synuclein. *Frontiers in Neuroscience*. 2017;**11**(172)

- [226] Zhang W et al. Exosomes in pathogen infections: A bridge to deliver molecules and link functions. *Frontiers in Immunology*. 2018;**9**(90):1-12
- [227] Messenger SW, Woo SS, Sun Z, Martin TFJ. A Ca(2+)-stimulated exosome release pathway in cancer cells is regulated by Munc13-4. *The Journal of Cell Biology*. 2018;**217**(8):2877-2890
- [228] Baixauli F, López-Otín C, Mittelbrunn M. Exosomes and autophagy: Coordinated mechanisms for the maintenance of cellular fitness. *Frontiers in Immunology*. 2014;**5**:1-627
- [229] Vlasov AV et al. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochimica et Biophysica Acta*. 2012;**1820**(7):940-948
- [230] Kooijmans SA et al. Exosome mimetics: A novel class of drug delivery systems. *International Journal of Nanomedicine*. 2012;**7**:1525-1541
- [231] Lai RC et al. Exosomes for drug delivery—A novel application for the mesenchymal stem cell. *Biotechnology Advances*. 2013;**31**(5):543-551
- [232] Zhuang X et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Molecular Therapy*. 2011;**19**(10):1769-1779
- [233] Alexander M et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. *Nature Communications*. 2015;**6**:7321
- [234] Gupta A, Pulliam L. Exosomes as mediators of neuroinflammation. *Journal of Neuroinflammation*. 2014;**11**:68
- [235] Yang Y et al. Increased anti-tumour activity by exosomes derived from doxorubicin-treated tumour cells via heat stress. *International Journal of Hyperthermia*. 2015;**31**(5): 498-506
- [236] Sun D et al. A novel nanoparticle drug delivery system: The anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Molecular Therapy*. 2010;**18**(9):1606-1614