Chapter

Exosomes: The Surreptitious Intercellular Messengers in the Body

Naveen Soni, Jitender Jangra, Megha Chaudhary, Gargi Nandi and Bhawana Bissa

Abstract

Exosomes are secret intercellular messengers in the body, carrying crucial information from different organs. Different cargos can be packaged in exosomes including DNA, RNA, and proteins. The type of exosomal cargo can vary according to the tissue type, its pathophysiological state, and circadian rhythm. Therefore, exosomes have an immense potential to be utilized for diagnostic purposes if the conundrum of their cargo can be understood. Recent advances in exosome isolation and characterization have made it possible to define disease-specific cargo carried by these tiny messengers. We attempt to highlight disease-relevant exosomal cargos for diagnostic purposes.

Keywords: exosomes, cancer, neurodegeneration, cardiovascular, miRNA

1. Introduction

Cells are the most unique and well-established micro-machines that assemble and make efficient metabolic molecules and pathways to maintain homeostasis. Exosomes are one of those cellular secretions that carry hidden information about proteins, RNAs, DNAs, and metabolites of secretory cells. Certain amounts of these molecules are packaged in the exosomes during their synthesis and secreted from the cell in a normal process. These exosomes are released in the extracellular environment and get captured by the nearby cells. Exosome content carrying information of parent cells makes the physiological changes in the recipient cell, making them an intercellular messenger of genetic and metabolic information.

Extracellular vesicles are the membrane-enclosed form of cell cargo with dynamic size, variety, and diversity. These can be distributed in three types based on the diameter of the vesicle; microvesicles (100-1000 nm), exosomes (30-150 nm), and apoptotic bodies (50-5000 nm) [1]. Exosomes were first recognized in rat ovum and algae in the 1950s [2, 3]. Soon after this, the detection of EVs was also done in plants and fungi [4, 5]. At that time EVs were not recognized well, instead, they were thought to assist in removing garbage from the cell. Later in 1996, Raposo declared that antigen-specific MHC-2 containing vesicles from B-lymphocytes induces an antigen-specific response in T-cells, clarifying that EVs are not garbage anymore [6]. EVs were also

discovered in bat thyroid follicular cells by Nunez and Gershon [7]. This was the first chapter to explain the proximity of multivesicular bodies near the limiting membrane, and their fusion with the membrane to release them into luminal space. EV secretion is the ancient feature, followed by archaea, prokaryotes, and eukaryotes that play a significant role in cell-cell communication [8, 9].

2. Exosome biogenesis

Exosomes like vesicles (ELV) or exosomes are synthesized in the endosomal compartments in a well-regulated way, and stress, mutation, and alteration in the microbiological environment may change the generation and secretion of exosomes. This regulation is maintained by the multiple proteins such as RAB, SNAREs, and cytoskeletal proteins [10]. Endosomes are synthesized by the invagination of the cell membranes. A naive endosome is non-judgmental with no decided fate and is called an early endosome. It either can fuse with the available endocytic vesicles containing cargo for export/degradation/recycling or can mature into late endosomes [11, 12]. Late endosomes are slightly more acidic than early endosomes, which might affect exosome production. A study by Logozzi has revealed that when cells are grown in acidic pH, the amount of exosome synthesis also increases as compared to the buffered medium [13]. Within the late endosome, the inward budding of the membrane synthesizes intraluminal vesicles that accumulate cytosolic content such as nucleic acid, proteins, metabolites, ions, and lipids. At this stage, the whole organelle containing ILV is considered as MVBs (Multi Vesicular Bodies). The pre-decided fate of MVB is not clear whether it will fuse with lysosome or autophagosome or exocytose to deliver in luminal space.

Exosomes are synthesized in the MVBs in the form of ILVs. Biogenesis of exosomes necessitates enrichment of CD9 and CD63 tetraspanin molecules and assembly of ESCRT (Endosomal Sorting Complex Required for Transport) complex at the site [14–16]. ESCRT is the preferred route of ILV formation but if ESCRT is necessary for cargo selection and exosome secretion is still controversial. ESCRT consists of four ESCRT protein complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. Another AAA ATPase Vps4 complex works together with the ESCRT-III to deform and amputee endosomal membrane [17]. These protein complexes show a high degree of cooperativity while sorting cargoes, vesicle budding, and MVB biogenesis [18]. The whole process of cargo selection, vesicle formation, and cargo incorporation occurs simultaneously. ESCRT-0 complex first initiates the recognition of microdomains on the endosomal membrane where ubiquitinated proteins are sequestrated. This occurs through the recognition by the HRS protein of ESCRT-0 to TSG101 of ESCRT-I. This initiates the invagination of the membrane, considering that all the proteins assigned for their secretion/degradation are clustered. ESCRT-0 complex interacts with the ESCRT-I and ESCRT-II and a wide-neck vesicle is formed inside the endosome [19]. Vesicle maturation marks the deubiquitination of clustered proteins. At this stage, ESCRT-0, ESCRT-I, and ESCRT-II units disassociate from the site and ESCRT-III assembles at the site. Snf7 protein, a unit of ESCRT-III, forms an oligomeric assembly and recruits ALIX (ALG-2 interacting protein X) at the site that stabilizes the ESCRT-III and promotes vesicle budding [20]. This complex narrows down the neck of the newly forming vesicle and then interacts with the AAA ATPase Vps4 complex, which is the key energy providing protein in releasing the vesicle from the endosomal membrane. Newly synthesized vesicle containing cargo now disassemble from the endosomal membrane and accumulate in the MVBs.

Another route for exosome production is the ceramide pathway, an ESCRT-Independent pathway. Microdomains present on the endosomal membrane are enriched with sphingomyelinases (SMases). These SMases cleave sphingomyelin lipid of the membrane, remove phosphocholine moiety and incorporate ceramide. These ceramides in the membrane induce lateral phase separation and amalgamate the microdomains of the membrane [21]. Consequently, a negative curvature is formed that promotes budding. Tetraspanins such as CD9, CD63, and CD81 are highly enriched in the exosome membrane and assist in protein sorting and exosome biogenesis. These tetraspanins containing microdomains are the specialized structure involved in an assortment of receptors and signaling molecules in the plasma membrane [22].

3. Exosome secretion

Rab protein is the largest family of small GTPases that governs the switch of GTP hydrolysis. More than 60 members of the Rab family are present in the intracellular membrane, serving as the main regulator of vesicle secretion [23]. Rab GTPases cover a major portion of membrane trafficking by its interaction with SNAREs, motor proteins, and coat proteins. The activation of GTPase activity is regulated by the GEFs (Guanine nucleotide exchange factors). The study also demonstrates that the interaction of SNARE with Rab induces the release of exosomes [24]. Rab proteins are the key molecules that determine the size of exosomes and regulate MVB docking at the plasma membrane, such as Rab27a, and intracellular distribution of MVBs for exosomal traffickings, such as Rab27b [25, 26]. Rab27a and Rab27b interact with their respective effector proteins, Slp4 and Slac2b, respectively, to transfer MVBs from the perinuclear to the periphery area of the cell [25]. Abolishing these interactions leads to decreased exosome release and inhibited breast cancer cell invasion and migration [27]. Additionally, some factors such as HSP90 and lysosome-associated protein transmembrane-4B (LAPTM4B) also transfer MVBs toward the periphery to promote their secretion [28, 29]. KIBRA interacts with Rab27a and enhances its retention, while some other GEFs such as MADD and Fam45a control exocytosis [26, 30, 31]. Rab11 and Rab35 are majorly involved in the endosome recycling pathway, and also assist in exosome secretion and cargo selection. Loss of function in Rab11 and Rab35 results in exosome accumulation in the cells [32]. But a similar study declaring that Rab11a and Rab7 remain uninvolved during the exosome biogenesis process is still controversial. The same study also shows that Rab7 enhances the release of exosomes containing Alix and syntenin in breast cancer cells but its knockdown does not affect exosome release in HeLa cells [25, 33]. Some small GTPases such as Rab2a, Rab5a, and Rab9a also increase exosome secretion [25]. These diversified functions of Rabs modulate the exosome biogenesis machinery and its secretion out of the cells. HRS, STAM1, and TSG101 silencing decrease the exosome release in dendritic cells [34].

4. Exosome cargo

The exosome content is solely dependent on the extracellular environment and intracellular metabolic activities that may vary at any stage of the cell. Exosomes are loaded with RNA, DNA, lipids, and proteins with different concentrations and types. This specificity can be changed from cell to cell even with the same environment. Many

studies are ongoing and have been done to get exosome content. For now, few databases are accessible to collect information about the exosome cargo. These are exoRBase, Exocarta, EVpedia, Vesiclepedia, EV-TRACK, and ExoBCD [35–42]. For now, >9700 proteins, >3400 mRNA, >2800 miRNA, and > 1100 lipid data in EVs have been identified [39].

a. DNA: A diverse nature of nucleic acid content is found in the secreted EVs but only a few cases have been studied where genomic and mitochondrial DNA of EV works in cancer detection [43, 44]. Exosome-gDNA-based liquid biopsies for colorectal cancer are often performed [45]. Mammalian cells often discard the mutated portions of DNA and some transposon elements that are harmful to the cell. This fragmented gDNA and mtDNA get accumulated in the cytosol and packaged in exosomes for their secretion out of the cell. A study on pancreatic cancer identified mutated p53 and KRAS DNA in the serum exosomes, revealing it as an important biomarker for early detection of cancer [43, 46, 47]. In the same studies, it was also identified that large double-standard gDNA fragments reflect the mutation status of the tumor, important for molecular mapping. Considering this, exosome DNA databases can be formed for early screening of diseases, DNA modifications, and evaluating drug resistance.

b. RNA (coding, non-coding): Exosomes are enriched with the RNAs specifically functional non-coding RNAs [48]. Out of them, certain miRNAs are found so frequently with high diagnostic potential. Overall, exosomes are enriched with tRNA, 18S, and 28S rRNAs but other RNA species such as mRNA, miRNA, Pre-miRNA, Y-RNA, tRF, lncRNA, sncRNA, *piwi*-RNA, circRNA, and vault RNA are also found [49–52]. RNAs in the EVs were found from as short as 200 bp to >4 kb in length with most of them containing 3'-UTR regions [53]. These RNAs remain protected with the vesicle lipid bilayer or are associated with some RNPs such as RNPs or lipoproteins (HDL and LDL) [54].

   Different mechanisms have been proposed for the loading of RNAs in the EVs. For example, specific sequences within the 3′ UTR act as "zip-code" to export certain specific RNAs in the EVs. These "zip-codes" are about ~25 nt in length, such as the binding site for the miR-1289 carries by another mRNA containing the "CTGCC" sequence on its stem-loop structure [55]. It has also been seen that certain miRNAs carrying four nucleotide sequence motif "GGAG" interact with the hnRNPA2B1, which enhances their sorting in the EVs [56]. In addition, post-transcriptional modifications of miRNAs determine their fate of retention, such as uridylated 3′-end of miRNAs are sorted into the EVs, while adenylated 3′-end keeps them to stay within the cell [57]. Another mechanism is based on the nSmase2 activity, which if, overexpressed, releases more amount of miRNAs by enhancing exosome production [58]. Apart from this, the role of argonaute protein in the loading of RNAs in EVs is still a controversial statement. Some studies support that the knockdown of argonaute protein decreases certain specific miRNAs in the EVs. Whether argonaute is found in the EVs or the MVBs or in the endosomes, is still a complex scenario in the EV research [59, 60].

c. Proteins: The information about the protein cargo within the EVs is still unclear due to the differences in cell types, culture conditions, and isolation procedures. Only a fraction of common EV proteins can be identified that are generally found

in the endosomal pathway, like Alix, tetraspanins, and some ESCRT proteins. In addition, proteins for exosome secretion such as Rab27a, Rab11b, and ARF6 are commonly found in the EVs. Interestingly, most of the EVs contain tetraspanins (CD9, CD63, CD81, CD82, CD86), antigen-presenting molecules (MHC-1 and MHC-II), transcription factors (Wnt, Notch, hedgehog), transport and fusion proteins (GTPase and flotillin), heat-shock proteins (Hsp20, Hsp27, Hsp60, Hsp70, Hsp90), cell-surface peptidases (CD13 and CD26), and signaling receptors like EGFR [36, 61–64]. Exosome composition is mostly decided by the cell type it is derived from. A drug-resistant cell secretes exosomes containing MDR-proteins such as ABCB1, ABCC2, ABCG2, and p-glycoproteins to enhance the tumor environment more resistant to drugs [65, 66].

d. Lipids: Exosomes are loaded with certain metabolic active lipids that regularly participate in exosome syntheses, such as sphingomyelins, ceramides, aminophospholipid, cholesterol, and phosphatidyl serine [66]. In addition, a high phosphatidyl serine ratio in the outer layer of exosomes may enhance their uptake by target cell [67]. Overall, the lipid composition of exosomes is ~80% similar to the parent cell but the amount of polyunsaturated glycerophosphoserine and phosphatidyl serine makes it more unique and different [68, 69].

e. Metabolites: Much research is ongoing to capture attention toward the metabolite composition of extracellular vesicles. By targeting urine, serum, or plasma vesicles, more promising results have already been revealed [70–72]. Mostly, exosome lipidomes have been quantified with glycerophospholipids, prenol and sterol lipids, glycerolipids, free fatty acids, and sphingolipids [73, 74]. Some studies also demonstrate that apart from lipids, few amounts of sugar, amino acids, organic acids, carboxylic acids, nucleotides, metabolic intermediates, carnitines, phenolic compounds, and vitamins are also present [75–77].

5. Exosomes in different biological fluids

Exosomes are very small molecules that formed within endosomes *via* different ESCRT-dependent processes [78]. Their sizes range from around 30 nm to 150 nm. These EVs are secreted into the various body fluids, such as blood, urine, saliva, breast milk, ascites effusions, nasal secretions, tears, amniotic, synovial, lymphatic, cerebrospinal, and seminal fluids by the various cell types found within the body, including red blood cells, B cells, T cells, mast cells, platelets, endothelial cells, fibroblasts, adipocytes, epithelial cells [79–85]. Exosomes present in them move through these fluids to other areas or interact with other cells to carry out a variety of biological functions, including the modulation of immune response [86, 87], antigen presentation [88, 89], and the transfer of RNA and proteins [90, 91], intercellular communication, non-classical protein secretion [92], and transmission of pathogenic cargo [93–95]. Exosomes are typically obtained from various body fluids using ultracentrifugation [96] based on the sedimentation principle, which yields a very pure exosomal fraction that is recognized as the gold standard. Size exclusion filtration [97] or chromatography [98] is a different procedure that involves filtering through a number of filters with pores smaller than 100 nm and then centrifuging (100,000 g) to concentrate the exosomes. The biological function and integrity of the exosome are maintained using this method. Using a solid support magnet or flow

cytometry, immune affinity capture [99] involves binding specific micro-beads to bio-fluids containing exosomes and separating the exosome-bound micro-beads from the bio-fluids. Exosome isolation is also done using kit-based techniques, such as the precipitation method ExoQuick [100] and the microfluidic technology (ExoChip) [101] based on the immunoaffinity methodology. The sample source and intended use of the exosomes may determine which of these various techniques and procedures to adopt, each of which has advantages and disadvantages of its own. Exosomes contain a variety of nucleic acids to perform various biological functions. The lipid bilayer's DNA, RNA, lipids, proteins, and metabolites keep them stable and allow for long-term storage. Even yet, the microenvironment and the type of cell to which an exosome is delivered determine what is contained within the cargo. As a result, the stability of different biomolecules within the exosome and their enrichment make them appropriate for a range of therapeutic and diagnostic uses. Exosome vesicles are primarily extracted from the serum, plasma, CSF, and urine and are the form that has been examined the most. As of now, exosome vesicles produced from particular fluids have more precise identification and validation than whole body fluid [78]. For instance, Kalra et al. [102] isolated EVs from plasma and demonstrated the depletion of highly abundant plasma proteins [103, 104]. As a result of their cargo and diverse features, exosomes are transformed by cells and may play a role in the progression of various diseases. As a diagnostic biomarker in the early detection and prognosis of diseases, these changed content (proteins or miRNAs) revealing distinct [78] profiles in exosomes are being different from the exosomes released by the normal/healthy cells. Another arm of exosomes is their therapeutic role for different purposes such as vaccination, biological targeting, and drug delivery tools, using a variety of therapeutic materials, including siRNA, antagomirs, g-RNA (siRNA), recombinant proteins, and anti-inflammatory drugs [105].

6. Exosomes in diseases

Exosomes in disease pathophysiology have recently attracted a lot of attention from researchers. Literature studies have shown that due to the potential ability of cell-to-cell communication among homozygous and heterozygous cell types, exosomes acts as a mediator for maintaining healthy physiological conditions [106]. In addition to their regular role, these exosomes are manipulated by the pathogen to infect the host cell activity [107] and act to potentiate stress and damage [106]. Exosomes have been discovered to play a role in the onset and progression of a number of diseases, including cancer, autoimmune disorders, neurodegenerative diseases [108], cardiovascular diseases, liver diseases [109], and genetic diseases, among many others.

6.1 Exosomes in tumor microenvironment, metastasis, and angiogenesis

Cancer is one of the oldest and deadliest diseases in the world. The ability of cancer cells to communicate with other cells is achieved primarily through exosome vesicles to maintain normal physiological conditions and trigger disease progression. These vesicles help cells to communicate between homotypic and heterotypic cells. In homotypic exosome transfer, exosome content and signaling capabilities allow cancer cells to progress and transmit cell growth, transformation, and survival signals to other cancer cells [110]. Various autocrine signaling pathways Akt/PI3K and MAP

kinase are involved in its progression [111]. As heterotypic exosome transfer involves all stages of cancer development and progression, tumor spread is driven by its local tumor microenvironment (TME). TME is composed of different cell types, including endothelial cells, fibroblasts, and immune cells. This tumor microenvironment enables a variety of cancer cell-derived exosomes, such as CAF-derived exosomes (CDE) and fibroblast-derived exosomes, to sustain proliferation, evade growth suppression, evade immune recognition, and activate invasion and metastasis cascades. It helps in regulation, resisting cell death, initiation of angiogenesis, promotion of cell proliferation, and deregulation of cell energetics through juxtracrine and paracrine signaling interactions [112, 113].

In cancer metastasis, primary tumor cells migrate to another part of the body where they multiply and form new tumors. There are various stages in this process: vascular invasion, extravasation, tumor latency, and formation of macro- or micrometastases [114]. The process of metastasis is modulated by EMT, ECM remodeling, activity of the immune system, and alteration in tumor micro-environment [115, 116]. However, exosomes play a significant role during metastasis, as it influences tumor roles and primarily contributes to the formation of the pre-metastatic niche that determines specific organotrophic metastasis [114, 117]. During the invasion, the primary tumor releases various factors (microRNAs, EGFR signaling ligands, EMT inducers, etc.) that promote invasion [118–120]. For example, miR-10b is transported and released by exosomes and promotes the metastatic properties of breast cancer cells [121]. Another, miR-23a inhibits E-cadherin synthesis in lung cancer and melanoma cells, thereby inhibiting the release of TGF-1 supporting EMT-promoting effects [50, 51]. EGFR signaling factors include ligands such as amphiregulin, tissue-type plasminogen activator, and/or annexin II, and significantly increase cancer cell invasion [122]. Exosomes secrete EMT inducers such as vimentin, snail, and twist in urothelial cell lines while reducing E-cadherin and catenin expression through the TGF-1 pathway [123] . These exosomes have properties that drive exosome organotropism in cancer cells, and ITG**α**6β4 and -**α**6β1 are associated with lung metastasis, and ITG**α**vβ5 is associated with liver metastasis. Related, ITGβ3 is related to brain metastasis [124]. Exosomes also exhibit stromal cell proliferation, cancer cell migration and survival, and ECM remodeling that increases tumor cell resistance to apoptotic signals. This, along with the effect of chemokines and growth factors, leads to the formation of a new microenvironment for cancer cells, immune cells, and other stromal constituents that is referred to as the PMN [122, 125, 126]. For the initiation of the metastatic process, an adequate blood supply to the tumor facilitates the entry of tumor cells into the bloodstream [127]. Thus, angiogenesis provides an opportunity for tumor growth by supplying cancer with oxygen, nutrients, and metabolite replacement [127]. Exosomes can transport various biomolecules such as microRNAs, DNA fragments, proteins, lipids, and even pharmacological compounds from donor cells to recipient cells [128]. Therefore, noncoding RNAs, especially long noncoding RNAs (lncRNAs) and microRNAs, play important roles in regulating angiogenesis [129]. In addition, exosomes can interact with target cells such as endothelial cells (EC) as well as immune cells to initiate and promote angiogenesis. The uptake of tumor-derived exosomes by normal endothelial cells activates angiogenic signaling pathways and stimulates new blood vessel formation [130]. Exosomes migrate to the cell periphery and invade advanced pseudopods. After complete remodeling, neighboring ECs likely transport exosomes to other ECs and other cells within the TME (tumor microenvironment) *via* nanoparticle structures [131].

6.2 Exosomes in neurodegenerative diseases

The majority of human neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and Huntington's disease, have aggregation of aberrant proteins as a common mechanism [132]. The existence of vesicles in the CSF has proven that these EVs are involved in the pathogenic spread of harmful proteins [132].

Alzheimer's disease is a neurological disorder caused by the disparate modification of Amyloid beta (Aβ) peptide and tau protein. Exosomes carry proteases, APP and its C-terminal fragments (CTFs-APP) that are caused by gamma and β secretase within the early endosomes, ultimately exports Aβ into the exosomes [133]. Exosomes provide a unique pathway for removing Aβ from cells. However, they make Aβ more prone to aggregation and, therefore, could endanger neighboring cells [134]. Another protein called TAU is crucial for accelerating tubulin assembly into microtubules and preserving their structural integrity. Tau's protein biological activity is compromised by hyperphosphorylation, which also results in defective microtubule stabilization and the formation of neurofibrillary tangles that impact neuronal connection and function [135, 136]. The mechanism of exosome-based release of Tau protein helps microglia to spread damaging tau protein [137, 138].

Parkinson's disease is mostly caused by an accumulation of clumped or misfolded alpha-syn nuclei, which affect the cells' ability to function as neurons [139]. Exosomes are thought to protect against neuronal cytotoxicity and prevent intracellular protein aggregation by excreting alpha syn nuclei outside of cells. This could result in an increase in the concentration of harmful alpha syn nuclei in extracellular space. The exosomes can take up both big and small alpha syn structures and cause various forms of downstream mediated toxicity from healthy neuronal cells [140]. These aggregates can kill the other target cells [141–145]. In addition to these, there are several exosomal miRNAs that contribute to the progression of PD pathogenesis. MiR-7 binding to the 3' UTR of SNCA mRNA suppresses transcription, which results in miR-7 loss. This loss of miR-7 is what causes greater -syn upregulation, aggregation, and dopaminergic neuron death in the brain of PD patients [146]. Another mi-RNA, miR-4639-5p has been upregulated, which negatively controlled the post-transcription of DJ-1 to cause significant oxidative stress and neuronal death in PD patients [147]. These all suggest a multi-functional role of exosomes in PD pathogenesis.

6.3 Exosomes in kidney diseases

The role of exosomes in acute and chronic kidney disease is highly specialized. Studies have shown that cell-to-cell communication between different regions of the kidney and organs amplifies kidney damage [148]. This exosome vesicle release contains proteins from different regions of the nephron fragment, including the thick ascending limb of the Henle loop, the distal tubule, and the collecting duct [149]. Due to their different origins, they have different protein content than their origin and serve as biomarkers for certain diseases [150]. The extracellular vesicles release from podocyte mediate communication between glomeruli and renal tubules, whereby alterations in communication outside the vesicles can affect podocytes and cause tubular damage/injury [151]. Studies suggest that there is upregulation of CD2AP mRNA and downregulation of Wilms tumor protein 1 (WT1) in extracellular vesicles are potential biomarkers of podocyte injury. This mechanism contributes to damage amplification, development of tubule-interstitial fibrosis, and progression of CKD [152]. In acute chronic kidney disease, urinary exosomes miRNAs reflect the state of

injury and fibrosis by the release of miR-9a, miR-16, miR-200a, and miR-141 [153]. A specific transcriptional repressor for activating transcription factor 3 (ATF3) was increased in sepsis-induced AKI [154]. In chronic kidney diseases such as diabetic nephropathy, there is a high glucose concentration in renal cells that cause changes in exosome composition and trafficking, further modifying and damaging intact cells [155]. Bioinformatics analysis revealed high levels of miR-133b and miR-342 in urinary exosomes of patients with diabetic nephropathy type 2 (T2DN) [156]. In addition, there are specific miRNAs such as miR-let-7i-3p, miR-24-3p, and miR-27b-3p, whose downregulation is involved in Wnt/β-catenin signaling, leading to T2DN pathogenesis [157]. Exosomes overexpress cellular repressors of multiple genes such as envoplakin, villin 1, prominin 1, and E1A-stimulated gene 1 (CREG1), causing autosomal dominant polycystic kidney disease (PKD) with abnormal morphological and proliferative changes [158, 159]. Circulating extracellular vesicles may lead to intra-organ crosstalk that shows an impact on autoimmune kidney diseases such as systemic lupus erythematosus, anti-phospholipid syndrome, and ANCA-associated vasculitis. For instance, circulating extracellular vesicles may encourage coagulation, thrombosis, and immune-mediated renal pathological conditions [160]. Placenta-derived extracellular vesicles carrying anti-angiogenic factors that are released into the maternal circulation in pre-eclampsia may cause proteinuria and glomerular endothelial dysfunction [161].

7. Exosomes in cardiovascular diseases

Cardiovascular diseases are major global diseases that affect the circulatory system [162]. Exosomes produced from the cardiac cells are one of the components in the body that keep cardiac under hypoxia and improve heart function [163]. These exosomes show changes in their states under various cardiovascular pathophysiology conditions and maintain homeostasis primarily during stress signals [164, 165]. Numerous diseases, such as cardiac fibrosis, ischemic heart diseases, heart failure, myocardial infraction, and cardiac hypertrophy, exhibit changes in cargo and protein content and serve as a biomarker for physiological changes [162]. It has been demonstrated that the pattern of fibroblast gene expression is regulated by cardiac cell-derived exosomes [166]. On external stimulation, cardiac fibrosis results in a sustainable remodeling of the extracellular matrix (ECM) through non-canonical Wnt and ERK1/2 pathways, as well as JNK pathways [167]. These pathways are promoted by the WNT-5a-enriched exosomes resulting in IL6 production and fibrosis [168]. Exosomes serve as intercellular communication (regulates intimal integrity) and myocardium remodeling in conditions such as ischemic heart disease and myocardial infarction respectively allowing injured cells to communicate with distant normal cells [162]. Exosomes derived from fibroblasts promote the RAS system and activate angiotensin II in cardiomyocytes that accelerate in cases of cardiac hypertrophy [169].

8. Diagnostic potential of exosomes

Exosomes are small EVs (Extra vesicles) of size 30-150 nm in diameter secreted by both normal cells and diseased cells into the different body fluids such as plasma, saliva, bronchial lavages, urine, and many others [170]. These fluids having exosomes contain different biomolecules including RNA, DNA, proteins for their intercellular

communication, and transportation [171, 172]. There is differential expression of exosomal RNA and proteins derived from normal cells and diseased cells [173]. This exosomal protein and nucleic acid emerged as next-generation biomarkers for different pathology conditions such as neurodegenerative diseases, cardiovascular, kidney diseases, cancer, and others.

8.1 Proteins and cargo as diagnostic marker

In cancer cells, the protein content of exosomes varies between healthy cells and diseases, and it resembles a variety of conditions associated with cancer, liver, kidney, and brain diseases [174]. Exosome-specific protein serves as a biomarker for disease pathology. For instance, distinct protein expression of different fluids acts as a biomarker. In breast cancer, serum-derived exosomes show enrichment of ADAM10, metalloprotease, CD9, Annexin-1, and HSP70 [175] proteins, and plasma-derived exosomes show diagnostic potential for fibronectin and developmental endothelial locus-1 (Del-1) [176]. In lung cancer, expression of CD151, CD171, and tetraspanin 8 is higher in serum exosome [177]. Glypican-1 (GPC1)-positive exosomes serve as potential biomarkers in early-stage pancreatic cancer [178] and CD26, CD81, S1C3A1, and CD10 could be used as a potential biomarkers for hepatic damage [179].

Apart from cancer, other diseases also have significant alteration in exosomes profile and lead to different expression of proteins act as a biomarker for diagnostic potential. In neurodegenerative diseases such as Parkinson diseases elevated expression of different proteins such as PrPc (glycoprotein) [180], DJ-1 [181] (plasma neural-derived), OxiDJ-1 [182] (urine-derived), and Tau Protein (neuron-derived) could be a marker for PD diagnosis. Other potential biomarkers such as a decreased expression of C1q derived from serum exosome and more of blood-derived Apolipoprotein A1 (Apo A1), clusterin, complement C1r subcomponent, and fibrinogen gamma chain exosomal expression levels in the plasma of PD subjects may serve as a biomarker for the diagnosis of PD [183, 184]. In Alzheimer's diseases, human serum-derived exosome shows an elevated expression of Cathepsin-D, LAMP-1, ubiquitinylated protein [185]. Downregulation of SNAP-25 [186] marks the synaptic loss during the progression of AD and HSP70 [185, 187] shows dysfunction and neurodegeneration.

The kidneys play a crucial role in the human body's homeostasis regulation and maintenance [188]. The release of exosomes from various parts of the kidney facilitates cell-to-cell communication that has an impact on the physiology of the kidney [189]. The proteins and nucleic acids contained by exosomes carry through the urine serve as a non-invasive diagnostic biomarker for renal diseases. For instance, the protein level of Fuetin-1 and AQP2 have been identified as potential biomarkers for acute kidney injury (AKI) [153, 190]. There is an increased amount of neutrophil gelatinase-associated lipocalin (NGAL) and activating transcription factor 3 used as a marker for early diagnosis in sepsis-induced AKI [154]. The non-invasive biomarker for PKD is demonstrated by the elevated expression of the urinary exosome proteins such as villin-1, periplakin, and envoplakin in ADPKD (autosomal dominant polycystic kidney diseases) [158]. However, increased expression of AQP-2 and AQP-5 in exosomes in chronic diseases like diabetic nephropathy can be used as a biomarker to diagnose T2DN [191].

8.2 Nucleic acid as diagnostic and prognostic biomarker

Exosomes secreted from diseased cells contain different biomolecules than the healthy ones. Therefore, the basic nucleic acid content also varies with the diseases

and mainly circulating microRNAs are being focused to carry out effective diagnosis and prognosis of numerous diseases [192]. For example, in breast cancer, the plasma-derived exosomes show the elevated expression of miR-1246 and miR-21 compared to healthy individuals [193, 194]. These serum-derived exosome shows miR-21 levels, which differentiate between metastatic and non-metastatic breast cancer [195]. Apart from these, upregulation of miRNAs, such as miR-223-3p, miR-16, miR-27a/b, miR-152, miR-199a-3p, miR-340, miR-376a, miR-410, and miR-598 [196–198], shows the presence of breast tumor. In non-small cell lung cancer (NSCLC), there is upregulation of different exosome miRNAs subset of 4 miRNAs (miR-378a, miR-379, miR-139-5p, and miR-200b-5p) and six miRNAs (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p) respectively [199, 200]. Other than this, plasma-derived miRNA-9 and miRNA-15 can distinguish a metastatic and aggressive state of tumor, thus having high potential as a diagnostic marker for NSCLC [201]. However, in the diagnostic marker for hepatocellular carcinoma, there is upregulation of miRNA-21 in the plasma, which distinguishes patients from healthy individuals [202]. On the other hand, the cancer malignancy in hepatoblastoma is mediated by a panel of miRNAs involving miR-21, miR-34a, miR-34b, and miR-34c in plasma which are verified as diagnostic and prognostic tool [203]. High levels of miRNA-10b, miR-21, miR-30c, and miR-181a and decreased let-7a levels are seen in pancreatic ductal adenocarcinoma patients as compared to a healthy individual [204].

In neurodegenerative diseases such as Parkinson's and Alzheimer's disease, blood and peripheral fluids also contain exosomes synthesized from nerve cells that are passed through the BBB (blood-brain barrier). These fluids show different miRNAs expression in patients and healthy controls. miRNAs from serum are the non-invasive and feasible approach to determining biomarkers for neurodegenerative disease. Exosomal miRNAs derived from plasma, CSF, and serum are being either upregulated or downregulated in different pathophysiological conditions. Certain serum-derived miRNAs such as miR-15a-5p, miR-18b-5p, miR-30e-5p, miR-93-5p, miR-106a-5p, miR-143-3p, miR-335-5p, miR-361-5p, and miR-424-5p are upregulated in comparison with healthy individuals and some of the miRNAs such as miR-15b-3p, miR-342-3p, and miR-1306-5p are downregulated in AD patients [205, 206]. CSF-containing exosomes also show potential diagnostic miRNAs such as upregulation of miR-125b-5p and downregulation of miR-16-5p and miR-451a [207]. These differently regulated miRNAs from different fluids improve the early onset and late-onset diagnosis and prognosis of AD. Similarly, in Parkinson's disease, several miRNAs derived from different fluids have differentially regulated miRNAs. Serum-derived exosomes have upregulated miR-24, miR-195, and miR-29a [208]. In plasma-derived exosomes, elevated level of miR-331-5p and let-7e-5p is observed. Some miRNAs like miR-10a-5p, miR-151a-3p, let-7f-5p, and many more are seen upregulated in CSF-derived exosomes [209]. Not only the upregulated miRNAs from different fluids show diagnostic potential but the downregulated miRNAs compared to healthy controls also act as diagnostic markers. For example, in PD patients, CSF-derived exosomal miRNAs show downregulated expression such as miR-27a-3p, miR-423-5p, miR-22-3p, miR-1, miR-22, miR-29, miR-374, miR-119a, miR-28 [210]. Some miRNAs such as miR-505 and miR-19b derived from plasma and serum respectively also show downregulation in PD patients [211].

Kidney diseases include AKI (acute kidney injury), chronic kidney diseases, diabetic nephropathy, polycystic kidney diseases, and various others. Exosomes isolated from urine contain differential biomarkers in form of microRNAs. In the AKI condition, urinary exosomes show various miRNAs for different conditions such as AKI progression including miR-16, miR-24, and miR-200c [212]. Also, miR-210 predicts AKI mortality

in ICU patients [213]. In sepsis-induced AKI, there is decreased expression of miR-376b, which acts as a potential biomarker for diagnosis [214]. Certain serum-derived exosomes reportedly decreased miR-24, miR-23a, and miR-145 expression in post-myocardial infarction AKI pathogenesis [215]. There are other serum-derived miRNAs that show a change in expression from healthy and AKI-diseased individuals including miR-101, miR-127, miR-210, miR-126, miR-26b, miR-29a, miR-146a, miR-27a, miR-93, and miR-10a [216]. Other kidney diseases, such as Diabetic Nephropathy, miR-192 is a master miRNA regulator of DN [212, 217]. Expression of miR-130 and miR-145 is upregulated, while miR-155 and miR-424 have reduced levels in diabetic patients with microalbuminuria, acting as a biomarker [218]. miR-415 derived from urinary exosome shows elevated expression in albuminuria and glomerulosclerosis and acts early diagnostic biomarker. miR-126 and the miR-770 family are derived from urine and blood as a promising biomarker for DN progression [212]. Although in diabetic patients, some Urine exosomal miRNAs including miR-192 and miR-21 show upregulated expression while reduced miR-30b levels which altered kidney function [219–222]. In type 2 diabetic nephropathy, the most upregulated miRNAs are MiR-34a and miR-320c which acts as a biomarker, and sediment miR-95 and miR-631 also reflect the severity and prognosis of type 2 DN [223–225]. Apart from these, certain other potential miRNAs biomarkers include miR-15b, miR-636, miR-34a, and miR-4534 in urine [226, 227] and miR-638 in serum. The ratio of albumin–creatinine shows an effect on miR-103a suggesting miR-103a as a dynamic biomarker reflecting pathological status and treatment response [228]. The role of EV miRNAs like miR-3907 upregulation in circulation predicts Autosomal Dominant Polycystic Kidney Disease progression [229]. Diagnosis shows other serum-derived miRNAs including miR-17 family members (miR-20a, miR-93, and miR-106a) show a significant decrease in expression after hemodialysis [230]. Apart from these, in some chronic kidney diseases such as hypertensive nephropathy, Lupus Nephritis, kidney immune diseases, and many others, the role of serum and urine-derived miRNAs show a prominent role in diagnosis, prognosis, and disease progression.

In cardiovascular diseases, circulating EVs miRNAs, miRNA-425, and miRNA-744 acts as a novel biomarkers for cardiac fibrosis [231, 232]. Also, miR-30d is associated with deleterious cardiac remodeling and the expression of fibrosis and

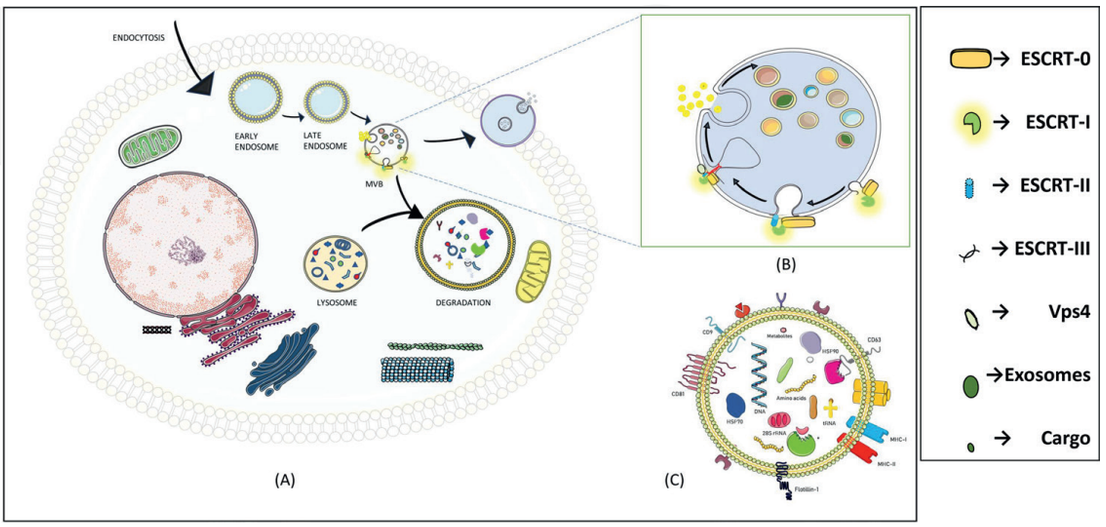

Figure 1.
(A) Multivesicular bodies can either merge with the lysosome or the autophagosome or be secreted out of the cell as a secretome. (B) Major pathways of exosome biogenesis (C) model structure of exosomes/EVs that carry certain proteins and receptors.

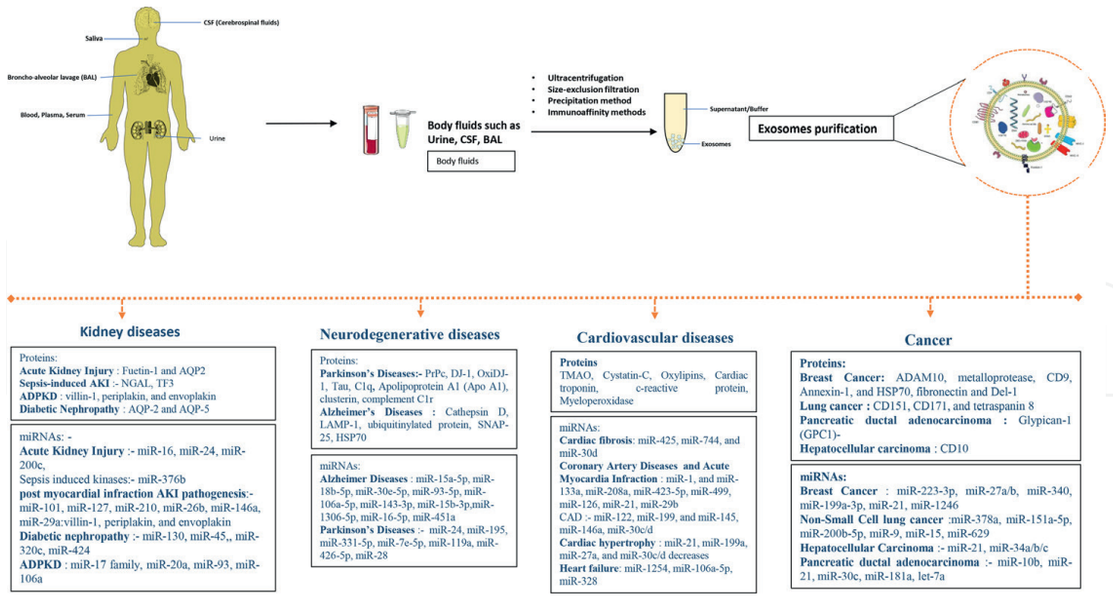

Figure 2.
Schematic representation of exosome isolation and diagnostic importance in different types of disorders.

inflammation-related genes [233]. In both coronary artery diseases and acute myocardial infraction, several exosomal miRNAs including miR-1, miR-133a, miR-208a, miR-423-5p, miR-499, miR-126, miR-21, and miR-29b show increased expression which potentially acts as a diagnostic biomarker as well as a prognostic marker for left ventricle remodeling [234–238]. However certain miRNAs including miR-423-5p [237], miR-499 [235], and miR-29b [239] essentially for AMI. miR-122 andmiR-199a [240] have elevated expression and miR-145 [241], miR-146a [242], miR-30c/d show downregulation that acts as diagnostic marker for CAD [243]. In contrast, miR-21, miR-199a miR-27a, and miR-30c/d show elevated levels, thus having a diagnostic potential of cardiac hypertrophy [244, 245]. However in heart failure diseases, the increased expression of miR-1254, miR-106a-5p, and decreased expression of miR-328 are other potential miRNA molecules apart from miRNA included in AMI, CAD, and cardiac hypertrophy, which act as diagnostic biomarker [246–248] (**Figures 1** and **2**).

Author details

Naveen Soni, Jitender Jangra, Megha Chaudhary, Gargi Nandi and Bhawana Bissa*
Department of Biochemistry, Central University of Rajasthan, Ajmer, Rajasthan, India

*Address all correspondence to: bhawana.bissa@curaj.ac.in

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