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Review

An overview of current knowledge in biological functions and potential theragnostic applications of exosomes

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ABSTRACT

Exosomes are cup-shaped structures, made of two lipid layers. Their size is in the range of 30–150 nm. Exosomes are excreted to the extracellular space and function in local and systemic cellular communication. Based on their primary origins, they can contain substantial amounts of RNA, protein, and miRNA; the horizontal transfer of these contents significantly determines the exosome's biological effects. The endosomal origins of exosomes can be deduced based on their surface protein markers. The use of exosomes as a diagnostic biomarker and therapeutic tool, has numerous advantages because they do not pose risks such as aneuploidy and transplant rejection. This - overview highlights the recent findings in exosome development and current knowledge in exosome-based therapies.

1. Introduction

Most living cells release a range of extracellular vehicles (EVs), which are 20–200 nm in size. These EVs are categorized according to their size, function and cellular origins, as microvesicles (MVs) (also known as shedding vesicles or ectosomes), microparticles, prostasomes (from the prostate gland), tolerosomes (from the intestinal epithelial cells) and apoptotic bodies, nanovesicles and exosomes (Gomez et al., 2018; Marote et al., 2016). The content of EVs depends on their origin. Nonetheless, they mainly contain bioactive molecules, such as nucleic acids, microRNA, lipids, and proteins; these molecules are delivered to the adjacent and distant cells (Marote et al., 2016). Thus EVs can modify the recipient cell's fate, function, and consequently modulate the surrounding microenvironment (Fatima and Nawaz, 2015). Microvesicles and exosomes have attracted more attention from researchers than other types of EVs. Exosomes are defined as derivatives of the endosomal system, with a cup-shape morphology and a size of 30–150 nm (Lener et al., 2015). They are secreted into the extracellular matrix through the fusion of multivesicular bodies with the cell membrane. Exosomes have been detected in all body fluids including blood, cerebrospinal fluid, urine, saliva, and breast milk, which makes their evaluation less invasive and more cost-effective (Yáñez-Mó et al., 2015). Exosomes are secreted by a myriad of cell types and they are named according to the cell type of origin. For example, vesicles secreted by the human prostate gland are referred to as Prostasomes, which are found in seminal or prostatic fluid (Aalberts et al., 2014). Also, microvesicles secreted by cancer cells, are termed Oncosomes, which contain oncogenes or oncogenic factors (Di Vizio et al., 2009).

Abbreviations: AD-MSC, adipose-derived MSC; Aex, ascite-derived exosomes; Ang II, angiotensin II; AT1/2R, angiotensin receptor types 1 and 2; AST, aspartate aminotransferase; BACE1, β-site APP cleaving enzyme 1; CCl4, carbon tetrachloride; CVDs, cardiovascular diseases; CRC, colorectal cancer; CK19, cytokeratin19; CTL, cytotoxic T lymphocyte; DEX, dendritic cell (DC)-derived exosomes; DLD, deterministic lateral displacement; ESCRT, endosomal sorting complexes required for transport; EVs, extracellular vesicles; GM-CSF, colony-stimulating factor; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; hucMSCs, human umbilical cord-MSCs; IGF1, insulin-like growth factor 1; ILV, intraluminal vesicles; LBPA, lysobisphosphatidic acid; MSC, mesenchymal stem cells; MVs, microvesicles; MVB, multivesicular body; NTA, nanoparticle tracking analysis; NKc, natural killer cells; NGF, nerve growth factor; NSCLC, non-small cell lung cancer; OPMDs, oral potentially malignant disorders; PCNA, proliferating cell nuclear antigen; PLP, proteolipid protein; PLD2, phospholipase D2; RAS, renin-angiotensin system; SEM, scanning electron microscopy; SDF1, stromal cell-derived factor 1; TLR, toll-like receptor; TDE, tumor-derived exosome; TGF-β1, transforming growth factor; TEM, transmission electron microscopy

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Tolerosomes have a vesicular structure; they carry MHC class II with bound antigenic peptides sampled from the gut lumen (Karlsson et al., 2001). Prominosomes are found in the luminal fluid of the embryonic neural tube and harbor the prominin-1 (CD133) stem cell marker (Marzesco et al., 2005). Exosomes contribute to extracellular communications in several manners. First, exosomes act as a nano-delivery system that transfers biological information specific to their progenitor cells, resulting in their specificity (Stremersch et al., 2016b). Second, exosomes' surface markers and their content makes them ideal biomarkers (Müller, 2012). Third, accumulating data received from exosome-based therapies have indicated that Mesenchymal stem cell (MSC)-derived exosomes are mediators of regeneration in different organs (Lai et al., 2010). Therefore, MSC-derived exosomes have been applied in the treatment of several diseases and in wound healing (Ko et al., 2015; Webber et al., 2015; Lou et al., 2017; Tan et al., 2014; Shabbir et al., 2015). Finally, exosomes can present the MHC-peptide complex and contribute to the activation of immune responses; hence, exosomes can be applied as vaccines (Zitvogel et al., 1998b). In this article, we review exosomes' roles and functions, their potential therapeutic application in several diseases, including in wound healing, and in anti-tumor immunotherapy. Moreover, exosome history, biogenesis, characteristics, and purification methods are also explained.

2. Exosome biogenesis

Exosome biogenesis begins with early endosomes. Throughout the life cycle of eukaryotic cells, small amounts of intracellular fluid are engulfed and small intracellular bodies, called early endosomes, are formed. Early endosomes fuse with endocytic vesicles and incorporate their content; the resulting vesicles may have various destinations (recycling, degradation, or exocytosis). Some of the early endosomes undergo a series of transformations in order to convert to the late endosome or MVB (Multivesicular body) (30–100 nm vesicles) that bud into the lumen of late endosomes or intraluminal vesicles (ILV) through different pathways (Kowal et al., 2014). (Fig. 1.A)

The best-characterized pathway that recognizes ubiquitylated proteins, based on the endosomal sorting complexes required for transport (ESCRT) (Trajkovic et al., 2008). ESCRT consists of four different protein complexes, ESCRT 0–3 and the associated AAA ATPase Vps4 complex. The ESCRT-0 subunit is responsible for cargo clustering in an ubiquitin-dependent manner. This subunit recruits ubiquitinated proteins and clathrin to promote internalization. Prior to the formation of intraluminal vesicles (ILVs), ESCRT-I and ESCRT-II subunits trigger the budding process and elevate the enzymatic de-ubiquitination of cargo proteins. The ESCRT-III subunit drives vesicle scission, and the accessory proteins (especially the VPS4 ATPase) act to dissociate and recycle the ESCRT machinery (Théry et al., 2001; Ha et al., 2016). Another pathway that has recently been shown is the ESCRT-independent pathway. The elimination of key ESCRT components leads to the defective formation of MVBs but does not lead to their complete absence, indicating that the ESCRT-independent mechanism exists. These processes include certain lipids, such as lysobisphosphatidic acid (LBPA), ceramides, tetraspanins, or proteins such as the heat shock proteins (Stuffers et al., 2009). The formation of vesicles in this pathway depends on the self-organization of lipid and cargo domains. Several lipids, such as proteolipid protein (PLP), cholesterol, phospholipase D2 (PLD2), are enriched in exosome membranes that are secreted after ESCRT inhibition. It seems that these lipids are essential in distinct cell types for exosome biogenesis in the ESCRT independent pathway (Kowal et al., 2014).

3. Functions of exosomes

The analysis of exosomal protein content shows that exosomes originating from different sources have different functions. At first, exosomes were originally described as a means for the disposal of redundant proteins in reticulocyte. Further studies revealed more functions for exosomes, such as being involved in the exclusion of harmful molecules from the cells, exchange of materials between cells, intercellular communication, propagation of pathogens, contribution to the immune system, antigen presentation, and etc.(Beach et al., 2014). Exosomes can also be used as diagnostic biomarkers, as they represent the physiological changes of the cells and tissues that they are derived from.

3.1. Exosomes in intercellular communication

Cell-to-cell communication involves intercellular and intracellular signals, which are transferred either by direct cell-to-cell contact or by secreted molecules (Camussi et al., 2010). EVs have been identified as a new means of communication because they horizontally transfer functional molecules, by carrying them to the adjacent cells (Cocucci et al., 2009).This leads to much more complex events, compared to the signaling mediated by a single ligand and receptor (Waldenström and Ronquist, 2014). Exosomes may bind to the target cell membranes, and as a result, they can acquire new surface molecules with novel adhesion properties. Furthermore, they can fuse with target cells to exchange proteins in the membrane and cytosol (cellular constituents of proteins, RNAs, and lipids) between two cell types (Franz et al., 2016; Piehl et al., 2013).

In total, the functions of exosomes depend on the cell/tissue that they are derived from. For instance, exosomes released by the central nervous system provide neural cell communication (Frühbeis et al., 2013).

3.2. Exosomes in the immune system

Exosomes act as modulators of the adaptive and innate immune responses. They participate in antigen presentation and in the distribution of antigens with MHC I - and MHC- II molecules (Sun et al., 2013). Exosomes can attenuate immune responses by inhibiting the differentiation of monocytes into the dendritic cells (DCs), improving the function of regulatory T cells, and suppressing the activity of natural killer and CD8+ cells (Liu et al., 2006). Also, Exosomes can stimulate the immune system through activation of natural killer cells (NK), B cells, and the increased survival of hematopoietic cells. Furthermore, exosomes obtained from human B cells have been shown to prompt antigen-specific MHC class I, II responses, along with other co-stimulatory molecules expressed in DC-derived exosomes (Ohno et al., 2013).

3.3. Exosomes in tumorigenesis

The intact membrane fragments or vesicles extracted from the malignant effusions and peripheral blood circulation of ovarian cancer patients were shown to express inherent markers to the tumor's membranes. Thus, it has initially been demonstrated that tumor-derived microvesicles contribute to tumorigenesis (Taylor et al., 1980). Cancer cells secrete exosomes 10-folds more than normal cells which are considered as the effective method of transferring metastatic information. The transfer of tumor-derived exosomes (TDE) content to the non-malignant cells triggers the activation of tumor formation and metastatic phenotype. In fact, this type of exosome has a significant effect on many tumor-associated events, such as metastasis, migration, proliferation, angiogenesis, drug resistance, and immune suppression (Shao et al., 2016). On the other hand, tumor antigens which are presented by DC-derived exosomes, trigger the cytotoxic T cell responses, which in turn leads to tumor rejection (Zitvogel et al., 1998a) (Fig. 1. B).

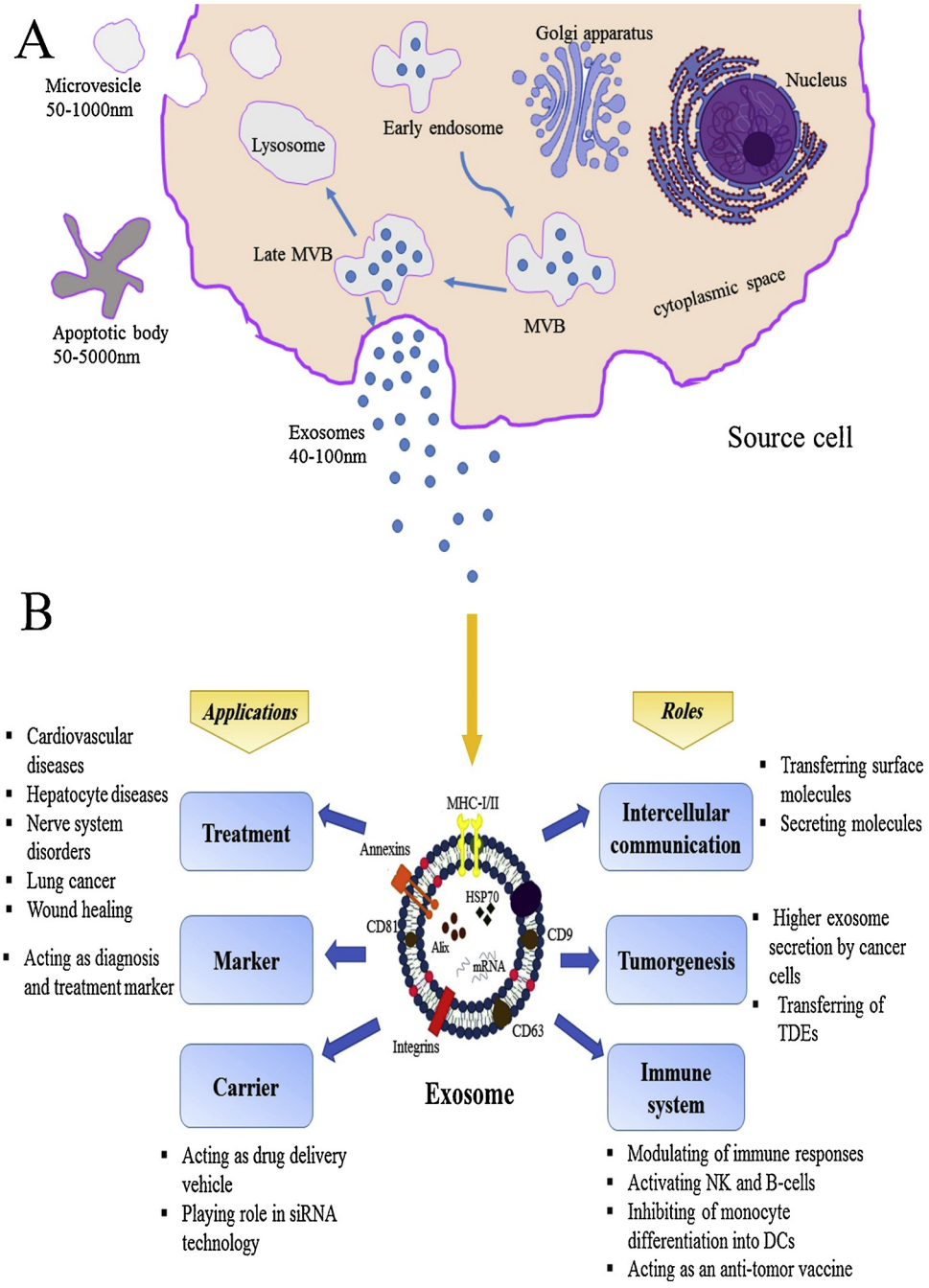

Fig. 1. The brief description of extracellular vesicles (EVs) biogenesis and their different size. Early endosomes are formed from plasma membrane budding and mature into the late endosome or multivesicular bodies (MVBs) which have two different fates. They can merge with the lysosome and be degrade or they can fuse with the plasma membrane to release exosomes outwardly (A). Exosomes mainly contain bioactive molecules, such as proteins, lipids, and mRNAs which are delivered to the other cells, so they can modify the recipient cell's fate (B).

4. Exosomes purification

EVs represent only a fraction of the cell's secretome, purified from a wide spectrum of body fluids and cell culture supernatants (such as T and B-cells, dendritic cells, platelets, mast cells, epithelial cells, endothelial cells, neuronal cells, and cancerous cells) (Rani et al., 2015). Different methods have been developed to extract exosomes; one of the most common procedures involves a centrifugation series to remove big debris and dead cells, followed by high-speed ultracentrifugation to pellet exosomes. In the Ultracentrifugation method, the separation of the exosomes is based on the density, size, and shape of the sequential separations. One of the benefits of this method is the lower costs and reduce risks of contamination. This strategy has some disadvantages, for example, it cannot distinguish between exosomes and other small vesicles or large protein aggregates. Another drawback is extensive personnel training, a need for large amounts of starting material, low yields of exosomes, inconsistent outcomes (exosome pellets or fractions are easy to lose), requiring the processes that are highly labor-intensive and time-consuming (Théry et al., 2002). Another technique is based on sucrose density gradient ultracentrifugation, which can purify exosomal fractions and precipitations. This method presents a poor specificity compared to size exclusion chromatography but provides a high yield of isolated exosomes that maintain their functions. The application of several approaches is recommended to increase the advantages and

decrease the issues associated with a single technique (Farooqi et al., 2017). Recently, the deterministic lateral displacement (DLD) pillar array technique has been proposed to extract exosomes, allowing for quantification in addition to on-chip sorting (Batrakova and Kim, 2015). The Immunoaffinity capture-based separation technique is based on specific interactions between the exosome membrane and the antibody. This method is useful for the isolation of specific exosomes and is performed with high purity. It is an expensive method that is applicable to cell-free samples (Batrakova and Kim, 2015). There is a distinction in the microfluidic separation method based on various characteristics of exosomes such as immunity, size, and density. High portability, high automation and integration, and low cost are the benefits of this method. The drawbacks of this method are the lack of standardization and large-scale clinical sample testing (Li et al., 2017).

As a way to scale up, simplify, and shorten exosome isolation, several companies developed the total exosome isolation kits. These allow for a simple and reliable concentration of intact exosomes from cell culture media and a range of body fluids (serum, plasma, urine, saliva). By tying up water molecules, the reagents force less soluble components (i.e., exosomes) out of solution, allowing them to be collected after brief, low-speed centrifugation(Invitrogen, life technology).

5. Biological characterization of EVs

There are different methods for characterizing EVs. Size and protein markers are the most important characteristics that have been studied. The EVs can be detected using transmission electron microscopy (TEM), scanning electron microscopy (SEM), and nanoparticle tracking analysis (NTA)(Sokolova et al., 2011). Furthermore, EVs can be further characterized by mass spectrometry, flow cytometry, immunoblotting, electron microscopy, and light-scattering methods. All of these techniques can measure the exosomal size (30–100 nm), and they can also determine the exosomal morphology (cup-shaped). Biochemical characterization can further reveal common surface, cytoplasmic, and cytoskeletal proteins that are characteristic of exosomes (Kesimer et al., 2009). The exosome's proteomic profile depends on its isolation method. The most abundant proteins in the EV subpopulation are tetraspanin (CD9, CD63, CD81, and CD82), MHC molecules, 14-3-3 proteins, cytosolic proteins such as heat shock proteins (HSPs), the ESCRT-3 binding protein Alix, and Tsg101(Witwer et al., 2013). It is noteworthy to mention, that CD63 and Tsg101 have only been detected in specific EV subgroups (Yoshioka et al., 2013); while CD9 and CD81 are the most identified proteins in EVs (Kim et al., 2013).

6. Applications

6.1. Therapeutic potential in clinical trials

Same as general exosomes, MSC-derived exosomes carry complex cargo, including nucleic acids, proteins, and lipids. They are involved in multiple biochemical and cellular processes, such as communication, immune regulation, bioenergetics, tissue regeneration, and metabolism (Lai et al., 2012). Because of their intercellular trafficking, they can act in paracrine or endocrine signaling by affecting neighboring and distant cells. Due to their potential in diagnostic applications, presence in different body fluids, and is less invasive and cheaper than other methods, MSC-derived exosomes are represented as ideal biomarkers and considered as a new therapeutic strategy (Stremersch et al., 2016a). (Fig. 2). More supplementary information is also provided in Table 1.

6.1.1. Exosomes as vaccines in anti-tumor immunotherapy

The idea of adopting EVs as an antitumor vaccine arose from a report which was published almost two decades ago. It was found that EVs contained MHC-peptide complexes are able to activate CD4 and CD8 T cells. In immune-competent mice, tumor cell peptide-pulsed dendritic cells induced the growing tumor rejection by activating tumor-specific cytotoxic T cells (Zitvogel et al., 1998a). This discovery resulted in phase I clinical trial for anti-non-small cell lung cancer (NSCLC) in the United States and an anti-melanoma phase I clinical trial in France (Escudier et al., 2005; Morse et al., 2005). In these trials, the conditioned medium of monocyte-derived DCs was used to recover EVs, then pulsed with antigenic peptides expressed by the patients' tumors (MAGE-A3 or A4). Due to the safety and feasibility of EV therapy, EVs from mature DCs were used for a phase II clinical trial (NCT01159288) of non-small cell lung cancer (Viaud et al., 2011). In another report, a phase I clinical trial of colorectal cancer (CRC) immunotherapy was conducted using Ascite-derived exosomes (Aex) in combination with the granulocyte-macrophage colony-stimulating factor (GM-CSF). The results showed that both therapies were safe and well-tolerated; however, Aex plus GM-CSF can induce a beneficial tumor-specific antitumor cytotoxic T lymphocyte (CTL) response. Since this polytherapy is safe and feasible, it can be a new strategy in the immune therapy of advanced CRC (Dai et al., 2008). Additionally, it has been reported that bacteria release EVs from their cell membrane. Therefore, EVs can be used as vaccine candidates against respective organisms. Thus, exosomes can increase the immune response to cancer cells as effective as bacterial-derived vesicles (Acevedo et al., 2014).

6.1.2. Exosomes in wound healing

Bone marrow MSC exosomes promote the expression of various wound healing-related growth factors, such as insulin-Like growth factor 1(IGF1), nerve growth factor (NGF), hepatocyte growth factor (HGF), and stromal cell-derived factor 1 (SDF1). They also activate multiple important signaling pathways in the wound healing process (e.g., Akt, ERK, and STAT3). Furthermore, the results of a study showed that MSC exosomes effectively increase the growth rate of wound fibroblasts. These findings suggest that these exosomes may mediate the wound healing process (Shabbir et al., 2015). Another important factor in wound healing is inflammation; controlling this response, without causing toxicity to the injured tissue remains challenging. The immunomodulatory effects of MSCs assists wound repair by releasing exosomes, which can provide a proper microenvironment through the horizontal transfer of exosomal microRNA. For example, the transfer of miR-21, miR-146a, and miR-181 to acceptor cells can regulate the expression of important immuno-modulators that fine-tune the TLR-induced NFκB signaling pathways and its downstream targets (Ti et al., 2016). In a skin-defect mouse model, it has been showed that umbilical cord-derived MSCs (uMSCs) can reduce myofibroblast accumulation and scar formation, which is associated with uMSC-derived exosomes enriched with specific miRNAs (miR-21, -23a, -125b, and -145). These miRNAs play key roles in suppressing myofibroblast formation by inhibiting the transforming growth factor-b2/SMAD2 pathway (Fang et al., 2016). In one study, human umbilical cord MSC-derived exosomes were used for the treatment of rat skin burn wounds. The enhanced re-epithelialization along with Cytokeratin19 (CK19), proliferating cell nuclear antigen (PCNA), and collagen I overexpression was observed. Additionally, when there was an interference in the expression of Wnt4 in hucMSC-Exosomes, their therapeutic effects were inhibited (Zhang et al., 2015). This suggested that activation of Wnt/ b-catenin by hucMSC-exosomes plays an essential role in exosome effects especially in cell proliferation.

6.1.3. Exosomes in liver disease

It has been shown in several studies that MSC-derived exosomes, in a multitude of animal models, have useful impacts on liver disease, including acute liver injury (drug-induced), liver fibrosis, and hepatocellular carcinoma (HCC) (Lou et al., 2017).

6.1.3.1. Liver fibrosis. MSC-derived exosomes are involved in ameliorating liver fibrosis by inhibiting both collagen production and epithelial-mesenchymal transition of hepatocytes. Furthermore, umbilical cord MSC-derived exosomes reduced the surface fibrous

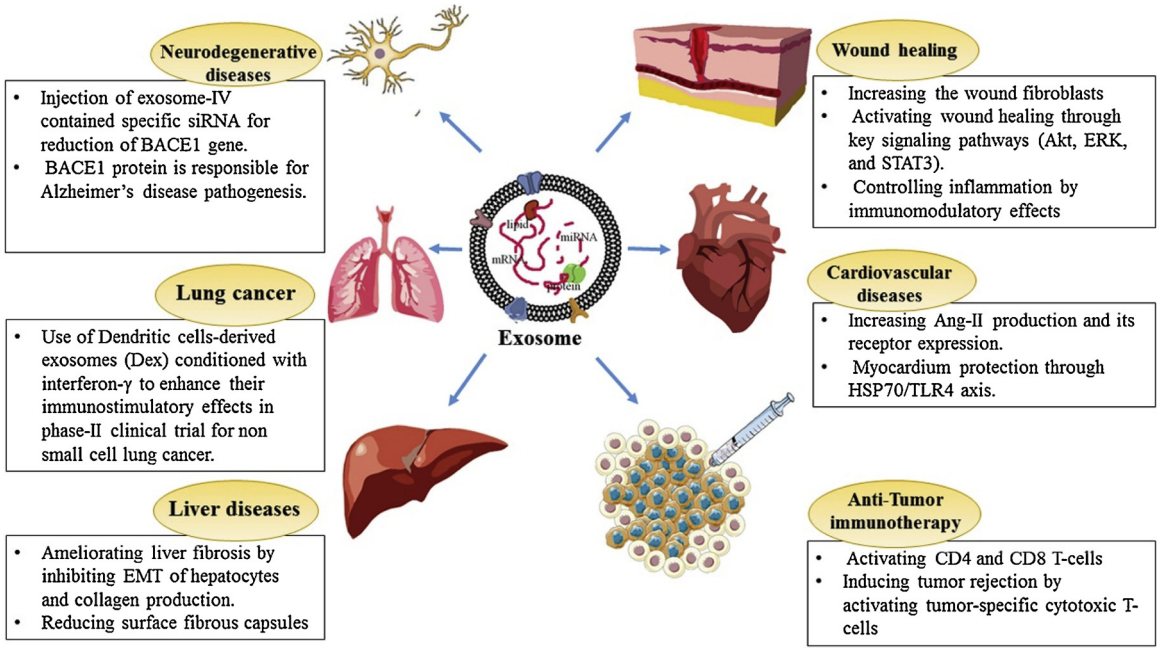

Fig. 2. Several therapeutic applications of exosome in various disease.

capsules and softened their texture, alleviated hepatic inflammation and collagen deposition in fibrotic liver. These exosomes also recovered serum aspartate aminotransferase (AST) activity significantly and decreased collagen type I and III, transforming growth factor (TGF)-β1 and phosphorylation Smad2 expression in a mouse model (Li et al., 2012). MicroRNAs are one of the important factors in liver regeneration. One study showed that exosomes containing miR-125b can decrease hepatic fibrosis by deterring the activation of Hedgehog signaling via the suppression of Smo expression resulting in reduced fibrosis (Hyun et al., 2015).

6.1.3.2. Acute liver injury. There are few reports on the therapeutic impact of MSC exosomes in acute liver injury. Recent research has demonstrated that exosomes derived from the HuES9.E1 MSC cell line, improved hepatoprotective activity in acetaminophen or H2O2-induced hepatocyte injury in vitro. However, in the following in vivo study on a mouse model of acute liver injury, induced by carbon tetrachloride (CCl4), increased hepatocyte proliferation, enrichment of PCNA, and higher cell viability were demonstrated (Tan et al., 2014). Another study on concanavalin-A-induced hepatitis indicated that transplantation of adipose-derived MSC (AD-MSC) exosomes can dramatically decrease serum levels of both aspartate aminotransferase and alanine aminotransferase as well as liver inflammation, liver necrosis, and the serum levels of proinflammatory cytokines (Lou et al., 2017).

6.1.3.3. Hepatocellular carcinoma (HCC). It has been recently shown that MSCs can be recruited from the bone marrow to the tumor microenvironment. Thus, they are considered as an appropriate candidate for tumor therapy (Barcellos-de-Souza et al., 2016). MSC-secreted paracrine factors delivered by EVs can contribute to tumor progression (Webber et al., 2015). Ko *et al.* demonstrated that AD-MSC exosomes suppressed the growth of hepatocellular carcinoma in a rat model. The exosome-treated rats showed partial tumor reduction, considerably smaller tumors, with a lower mean volume ratio, and higher apparent diffusion coefficient compared to the controls (Ko et al., 2015).

6.1.4. Exosomes in cardiovascular diseases (CVDs)

Exosomes contribute to cardiovascular diseases by communicating between cardiac fibroblasts, cardiomyocytes, and endothelial cells, through delivering biological information (Hirsch et al., 2013). Such results have been observed by Ronquist et al. who transfected exosomes containing mRNA from the cardiomyocyte into the fibroblast cells, which changed the gene expression (Waldenström et al., 2012). In addition, cardiac fibroblast exosomal miRNAs act as paracrine-acting RNA molecules to induce different effects through specific gene inhibition. For instance, Bang et al. showed that miR-21-3p induces cardiomyocyte hypertrophy by inhibiting sorbin, as well as other genes (Bang et al., 2014). Therefore, several exosomal genes and miRNAs, have already been identified in CVDs.

6.1.4.1. Heart failure. The chronic activation of the myocardial renin-angiotensin system (RAS) induces cardiac hypertrophy and a higher level of angiotensin II (Ang II). In one study, Lyu et al. demonstrated that Ang II treatment can potentially increase the production of exosomes from cardiac fibroblasts. Next, exosome-mediated upregulation of angiotensinogen, renin, Ang II receptor types 1 and 2 (AT1/2R), and downregulation of angiotensin-converting enzyme 2, increased RAS in cardiomyocytes. These results led to elevated levels of Ang II production in the aforementioned cells, which intensifies the Ang II-induced pathological cardiac hypertrophy (Lyu et al., 2015).

On the other hand, endothelial cell-derived exosomes containing mir-214 act as paracrine signaling mediators. These exosomes affect the ECs function and the crosstalk between them, which can enhance angiogenesis by stimulating the vessel formation and suppressing the ECs senescence (van Balkom et al., 2013). However, exosomes enriched with other miRNAs such as miR-125a, miR-21, and miR-135b have been found to change these miRNAs' target gene expression and promote angiogenesis in ECs (Umezu et al., 2014; Liang et al., 2016; Thuringer et al., 2016; Liu et al., 2016).

6.1.4.2. Myocardial ischemic injury. miRNAs have also been found in the ischemic heart. As an example, the higher amount of miR-133a and miR-1 have been observed to be localized in injured myocardium-derived exosomes, which can be delivered to the neighboring cells (Kuwabara et al., 2011). Moreover, in one study by Vicencio et al., the importance of exosome surface factors has been demonstrated. They identified a pro-survival signaling pathway in cardiomyocytes involving toll-like receptor (TLR4) and several kinases, leading to activation of

Table 1
Therapeutic application of several types of exosomes.

Therapeutic Application	disease	Exosome Source	Mechanism and result	Ref
Anti-tumor immunotherapy	Advanced non-small cell lung cancer (phase I clinical trial in the United States)	(Dex)	Vaccinating cancer patients (NSCLC) with tumor expression of MAGE-A3 or A4 with peptide-pulsed Dex. DEX therapy was well tolerated and the production of the DEX vaccine was feasible.	(Morse et al., 2005)
	Advanced NSCLC (phase II clinical trial in France)	(Dex)	Interferon-γ conditioning of the dendritic cell to induce the expression of CD40, CD80, CD86, and CD54 on Dex. Providing immediate and powerful peptide-dependent CD8(+) in vitro and in vivo T-cells.	(Viaud et al., 2011)
	Anti-melanoma, (phase I clinical trial in France)	Autologous DC-derived exosomes	Using autologous exosomes pulsed with MAGE 3 peptides for the immunization of stage III/IV melanoma patients.	(Escudier et al., 2005)
	colorectal cancer,(phase I),	(Aex)	Aex plus GM-CSF induces a beneficial tumor-specific antitumor CTL response.	(Dai et al., 2008)
Liver disease	Liver fibrosis: Mouse model	Human umbilical cord MSC-derived exosomes	Inactivation of TGF-β1/Smad signaling pathway by reducing collagen type I/III and TGF-β1, and phosphorylation of Smad2. Decreasing the surface fibrous capsules and softening their textures in fibrotic liver.	(Li et al., 2012)
	Acute liver injury: CCl4-induced acute liver injury/mouse model (C57BL/6)	HuES9.E1 MSC-derived exosomes	Increasing the proliferation of hepatocyte along with enrichment of PCNA and high cell viability.	(Lou et al., 2017)
	Hepatocellular carcinoma: rat model	AD-MSC exosomes	Promoting NKT-cell antitumor responses. Facilitating HCC suppression and low-grade tumor differentiation in exosome-treated rats.	(Ko et al., 2015)
Cardiovascular disease	Heart failure Cell culture and Mice model	Cardiac fibroblast-derived exosomes	Ang II stimulates CFs to release exosomes. Increasing Ang II production and its receptor expression in cardiomyocytes. Intensifying AngII-induced pathological cardiac hypertrophy.	(Lyu et al., 2015)
	Myocardial ischemic injury	An exosome-rich fraction from the blood of adult rat and human.	Mediating the myocardium protection through the HSP70/TLR4 axis.	(Vicencio et al., 2015)
Wound healing	In vitro wound healing	Human bone marrow MSC exosomes	Activating several signaling pathways essential to wound healing (Akt, ERK, and STAT3). Inducing the expression of a number of growth factors: (HGF), (IGF1), (NGF), and (SDF1).	(Shabbir et al., 2015)
	skin-defect mouse model	Umbilical cord- MSCs derived exosomes	Reduction of myofibroblast accumulation and scar formation by enrichment of exosomes with specific microRNAs (miR-21, -23a, -125b, and -145). Inhibiting the transforming growth factor-b/SMAD2 pathway.	(Fang et al., 2016)
	Rat skin burn model	Human umbilical cord MSC-derived exosomes	Enhancing re-epithelialization upregulation of CK19, PCNA, and collagen I expression.	(Zhang et al., 2015)

Abbreviations: Adipose-derived MSC (AD-MSC), Ascite-derived exosomes (Aex), Angiotensin II (Ang II), Carbon tetrachloride (CCl4), Cytokeratin19 (CK19), Cytotoxic T lymphocyte (CTL) Dendritic cell-derived exosomes (Dex), Colony-stimulating factor (GM-CSF), Hepatocyte Growth Factor (HGF), Insulin-Like Growth Factor 1 (IGF1), Natural Killer cells (NKc), Nerve Growth Factor (NGF), Non-small cell lung cancer (NSCLC), Proliferating Cell Nuclear Antigen (PCNA), Stromal cell-derived factor 1 (SDF1), Toll-like receptor (TLR).

the cardioprotective HSP27. In fact, the HSP70 in the plasma of exosomes mediate myocardium protection through the HSP70/TLR4 axis (Vicencio et al., 2015).

6.1.5. Neurodegenerative disorders

In relation to neurodegenerative disorders, Emmanouilidou et al. demonstrated that exosomes secrete α-synuclein, which is one of the involved factors in Parkinson's disease pathogenesis (Emmanouilidou et al., 2010). Furthermore, proteome studies have revealed that exosomes contain a variety of neurodegenerative disease related cytosolic proteins, with regard to their intracellular origins. These exosomes determine the involved mechanism of their release into the extracellular space. Moreover, proteins and biomarkers can be extracted from circulating exosomes and also even the probable mechanism of neuropathologic lesion spreading can be identified (Alvarez-Erviti et al., 2011a; Emmanouilidou et al., 2010). In one study, Alvarez-Erviti et al. utilized immature murine dendritic cell-derived exosomes and modified them. The final exosomes were contained specific membrane proteins and loaded with specific siRNA for the β-site APP cleaving enzyme 1 (BACE1) gene which is one of the proteins responsible for Alzheimer's disease. After exosome intravenous injection, the exosomes moved to the microglia, neurons, and oligodendrocytes of the mouse brain and decreased the BACE1mRNA and protein (Alvarez-Erviti et al., 2011b). These findings verify the potential of exosome-mediated RNAi technology in other neurodegenerative diseases by targeting their key mediators.

6.2. Exosomal miRNAs have both therapeutic and biomarker potentials

Exosomal miRNAs can be used as novel therapeutic treatments against several human diseases, including cancer, virus-induced, and parasitic diseases. A major limitation of miRNA therapy is the lack of an efficient cell-specific gene delivery system. In comparison with several gene delivery systems, exosomes provide unique advantages. First, exosomes can affect cell function by carrying biological information such as miRNA. They can also deliver biological information in the form of miRNA to specific cell types, through receptor-mediated binding. Second, exosomes have less off-target effects, due to their rapid absorption by recipient cells (Mittelbrunn et al., 2011). In a serial study, a group of researchers indicated that the transfection of miR-146b reduces migration, invasion, and viability of glioma cells. Moreover, miR-146b suppresses the malignant phenotype through EGFR inhibition (Katakowski et al., 2010). In one study, marrow stromal cell (MSC) exosomes were used as a vehicle for the delivery of miR-146b, which has anti-cancer effects. After the intra-tumor injection of these exosomes to a rat tumor model, the growth of xenograft glioma was significantly reduced (Katakowski et al., 2013). Furthermore, due to the different exosomal miRNA profile in patients and healthy individuals, they have attracted attention for their potential in the diagnosis of the pathological state and tumor development process (Michael et al., 2010). Another therapeutic application is in oral potentially malignant disorders (OPMDs). Genetically modified MSC-derived exosomes enriched with miR-185 diminished inflammatory response, inhibited cell proliferation and angiogenesis, and induced cell apoptosis, introducing them as a novel therapeutic approach for OPMD (Wang et al., 2019). Therefore, the miRNA levels in a certain stage of diseases or in circulating tumor-derived exosomes can be used as biomarkers. This can clarify the pathological disease states, mechanisms involved in the pathogenesis of diseases, and the potential treatment (Taylor and Gercel-Taylor, 2008).

6.3. Exosomes as drug carriers and delivery vehicles

Exosomes can be efficiently used as drug delivery vehicles that can pass through the blood-brain barrier. They are able to carry a substantial amount of therapeutic cargo to qualify as drug delivery vehicles. It is important to deliver interfering RNAs via exosome due to the low stability of RNAs and their fast degradation in the systemic circulation (Ozpolat et al., 2014).

The same methodology was used to induce both gene knockdown and reduce the viability and proliferation of fibrosarcoma cells by delivering RAD51- and RAD52-siRNA (Shtam et al., 2013). Down-regulation of both GAPDH (housekeeping gene) and BACE1 gene, was observed significantly in neurons following targeted delivery of siRNA-enriched exosomes (Alvarez-Erviti et al., 2011b).

7. Advantages and disadvantages of exosome

As we mentioned before, exosomes contain biological information such as DNA, protein, mRNA, and miRNA. Exosomes can deliver such information to the recipient cells, and if they carry cell-specific factors, they can possibly render information to the target cell. Thus, in spite of the distance between cells, they facilitate communications between them. Since they can be released into the body fluids, they carry and exchange information between different organs. Furthermore, by examination of the exosomal miRNAs, the diagnosis of disease and its involved mechanism as well as potential treatment can be obtained. of note, exosomes are less complex and they are smaller than routine MSCs, so their production and storage are more convenient (Katsuda et al., 2013). While exosomes can be used as an effective therapy for the delivery of different synthetic and biological molecules in cellular therapy of various diseases, there are still several challenges to optimize them. For instance, their characterization, quantification, and potency should be examined for clinical use. A higher amount of exosomes would be required to fulfill the equivalent impact of using MSCs, according to preclinical research so large-scale production techniques should be created (Phinney et al., 2015). Their large-scale production by bioreactors requires efficient techniques through expansion and immortalization. Furthermore, MSCs are heterogenic cells, thereby their exosomes are heterogeneous that may lead to a different effect on the target cells. In addition, MSC preconditioning with hypoxia condition, starvation, and inflammation to stimulate exosome release, can alter the content of exosomes. Taken together, these data suggest that exosome-based therapy requires a more concise insight. For this, establishing a gold standard for exosome characterization and preparing suitable preclinical animal models to obtain safety data would be promising.

8. Conclusion

Since exosomes can produce long-term immunity and naturally carry intercellular molecules and drugs, they have become therapeutic carriers. Extracted exosomes that have been characterized in various ways, can be used to treat different types of diseases but this process is still in clinical trial studies. In the near future, it will be expected that their usage in medical centers will be routine due to the improvement of the delivery methods and acquiring more knowledge about exosomes.

Declaration of Competing Interest

The authors declare no competing interests.

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