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The current landscape of the mesenchymal stromal cell secretome: A new paradigm for cell-free regeneration

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Abstract

The unique properties of mesenchymal stromal/stem cells (MSCs) to self-renew and their multipotentiality have rendered them attractive to researchers and clinicians. In addition to the differentiation potential, the broad repertoire of secreted trophic factors (cytokines) exhibiting diverse functions such as immunomodulation, anti-inflammatory activity, angiogenesis and anti-apoptotic, commonly referred to as the MSC secretome, has gained immense attention in the past few years. There is enough evidence to show that the one important pathway by which MSCs participate in tissue repair and regeneration is through its secretome. Concurrently, a large body of MSC research has focused on characterization of the MSC secretome; this includes both soluble factors and factors released in extracellular vesicles, for example, exosomes and microvesicles. This review provides an overview of our current understanding of the MSC secretome with respect to their potential clinical applications.

Keywords

cell therapy; exosomes; mesenchymal stromal cells; regeneration; secretome

Introduction

Mesenchymal stromal/stem cells (MSCs) were first found to co-exist in the bone marrow (BM) with hematopoietic stem cells (HSCs). Multiple properties of MSCs, including self-renewal, colony formation, phenotypic expression pattern and differentiation potential [1],

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attract therapeutic attention (Figure 1). It has also been shown that the MSCs are involved in several physiological and pathological processes, including tissue homeostasis, aging, tissue damage and inflammatory diseases [2,3].

Current knowledge on MSCs for repair and regeneration (preclinical and clinical studies)

When supplied exogenously, MSCs promptly respond to stress or injury in a manner that is very similar to how the adaptive and innate immune system cells respond to pathogen exposure or apoptosis. This capacity of MSCs is ascribed to their ability to respond to changes or requirements of the milieu through transcriptional regulation and translation of appropriate responding mediators that influence the milieu for repair, control of inflammation, regeneration, remodeling and cellular recruitment. The repair process involves regulating extracellular matrix deposition, collagen synthesis, fibroblast proliferation, platelet activation, fibrinolysis and angiogenesis. The immune process often involves suppressing T cells, activating macrophages and potentially recruiting neutrophils.

Recent studies have demonstrated that MSCs are inept for immunosuppression and become potentially immunosuppressive on stimulation [3]. Immunomodulatory properties of MSCs are one of their most attractive attributes for repair and regeneration because they can alter the secretome profile of dendritic cells, resulting in favorable changes in the microenvironment [4]. It has been reported that MSCs inhibit T-cell production and immunoglobulin (Ig)G secretion of B cells from BXSB mice used as an experimental model for human systemic lupus erythematosus [5,6]. Intracardiac allogeneic porcine MSCs elicit an immune response despite their low immunogenic profile *in vitro*, raising questions regarding the immune-privileged status of MSCs [7]. Intravenous (IV) injection of MSCs in rats leads to the formation of allo-antibodies that were sufficient to reduce survival of subsequently injected allogeneic MSCs [8]. On the basis of these findings, a thorough understanding of anti-donor immune responses elicited by allo-MSCs was emphasized [9] in a recent study.

MSCs isolated from BM (BM-MSCs) and adipose (ADSCs) exhibit specific differences at transcriptional and proteomic levels according to their tissue origin and functional differences with respect to their adipogenic, osteogenic and chondrogenic differentiation potential [1,10]. It has been shown that the ADSCs expressed higher levels of insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor-D (VEGF-D) and interleukin-8 (IL-8) compared with BM-MSCs, whereas other factors such as nerve growth factor (NGF), VEGF-A, basic fibroblast growth factor (bFGF) and angiogenin were expressed at comparable levels among them [11]. Thus, MSCs isolated from various sources may exert differences in their angiogenic potency. MSCs could also be an attractive cellular source for brain disorders, owing to the production of several neurotrophic factors such as brain derived neurotrophic factor (BDNF), nerve growth factor (NGF) or glial derived neurotrophic factor (GDNF) [12]. They can protect neurons against apoptosis [13] and can slow disease progression in models of Huntington disease [14]. In addition to preclinical trials in animals, several clinical trials (phase I/II) have endorsed the safety and efficacy of human MSCs (hMSCs) for myocardial infarction [15], multiple sclerosis [16], kidney transplantation [17], acute ischemic stroke [18] and Parkinson's disease [19]. Our group has recently shown that MSCs could be effective for the metabolic disorder type II diabetes in STZ-induced diabetic

mice without immunosuppression [20]. Furthermore, allogeneic MSCs have been demonstrated to be safe and efficacious for critical limb ischemia (CLI) in a phase I clinical study [21].

Influence of MSC niche

Stem cell niche is nothing but the specialized microenvironment in which most of the adult stem cells reside, including BM-MSCs. Tissue injury produces specific signals that activate stem cells for repair and regeneration of the damaged/lost tissue. In addition to tissue-specific adult stem cells that are primarily responsible for regeneration processes, BM-MSCs have been identified as important cell source that contribute toward regeneration of other tissues via paracrine signaling. MSCs present in the bone marrow niche can modulate the immune system and secrete certain trophic factors, which in turn help in regeneration [22].

These BM-MSCs stay in the bone marrow niche via series of interactions between the C-XC chemokine receptor 4 (CXCR4) and stromal cell-derived factor-1 α (SDF-1 α) [23]. On injury, these MSCs are reported to migrate to tissue sites via trophic factors. It has been showed that the released Substance P (SP) acts as a neurotransmitter and neuromodulator, was able to mobilize MSC-like cells from bone marrow into peripheral blood and subsequently to corneal tissues and participate in the repair process after corneal injury [24]. Several clinical trials used granulocyte-colony-stimulating factor (G-CSF) because it was known to accelerate mobilization of MSCs to the injured/damaged site [25]. Monocyte chemotactic protein-3 (MCP-3) recruits MSCs via activation of CC chemokine receptors into the myocardial infarction site [26]. Also, galanin, a neuropeptide, facilitates the migration of BM-MSCs by activation of the galanin receptor [27]. These studies indicate that there are several signaling pathways that regulate proliferation and mobilization of BM-MSCs from the niche environment either alone or in combination for effective tissue regeneration.

Migratory capability and homing of MSCs

Recently, Karp and Teo [28] defined MSC homing as the “arrest of MSCs within the vasculature of the respective tissue,” followed by transmigration across the endothelium. Chemokines, cytokines and growth factors released on injury provide migratory cues for systemically or locally administered stem cells. The cues induce activation of endothelium and subsequent adhesion of stem cells and transmigration into tissues to exert the therapeutic effects [29,30].

The interactions of SDF-1 α and CXCR4 were found to mediate the trafficking of transplanted BM-MSCs in a rat model of left hypoglossal nerve injury [28]. Inflammatory cytokines increase the production of matrix metalloproteases (MMPs) in MSCs, resulting in a strong stimulation of chemotactic migration through the extracellular matrix [31]. MSCs migrate in response to variety of growth factors and cytokines including platelet-derived growth factor (PDGF)-AB, transforming growth factor (TGF)- β 1, tumor necrosis factor- α (TNF- α), and SDF-1 α ; these cells showed enhanced motility when stimulated with TNF- α [32]. These findings establish the fact that better recovery of damaged tissues via cell therapy demands sufficient recruitment of cells to the target/affected tissue to promote

regeneration, mitigate inflammation and enhance angiogenesis. Nonetheless, MSC-driven homing activity to sites of neovascularization is a complex process that depends on a timely and spatially orchestrated interplay between chemokines, chemokine receptors, intracellular signaling, adhesion molecules and proteases. Thus, further elucidation of the molecular mechanisms of MSC homing to the site of injury is essential for the development of new therapeutic strategies and also to improve the efficacy of cell-based therapies.

Harnessing and mapping the MSC secretome

Exosomes are one of several groups of secreted vesicles, which also include microvesicles, ectosomes, membrane particles, exosome-like vesicles or apoptotic bodies [33]. Exosomes (40–100 nm) can be distinguished from microvesicles (MVs) (100–1000 nm) and apoptotic bodies (1–5 μ m) by size and morphology [34]. Unlike MVs, which are derived from plasma membrane shedding, exosomes are secreted by multivesicular bodies [35] (Figure 2). Scientists worldwide are currently trying to identify the role of exosomes and MVs present in the cell secretome. Exosomes isolated from stem cells were shown to be the naturally occurring nanospheres composed of a lipid bilayer like a liposome. These exosomes protect, transport and deliver a variety of small molecules released from stem cells to the surrounding cells [36]. Extracellular vesicles (EV) were first discovered in sheep reticulocytes during maturation in the 1980s [37]. Thereafter, many cell types were found to secrete EVs, including B cells, dendritic cells, mast cells, T cells, platelets, Schwann cells, tumor cells and sperm [38]. They are also found in physiological fluids such as normal urine, plasma and bronchial lavage fluid [39]. A recent study showed that MSCs do secrete EVs and contribute to the improvement of impaired neurological functions and enhanced angio-neurogenesis [40].

Exosomes

The exosomes tend to be homogenous in size (40–100 nm; Table 1) and are released by p53-regulated exocytosis with a density of 1.13 to 1.19 g/mL in a sucrose solution and can be sedimented by centrifugation at 100,000 *g*, which is dependent on cytoskeleton activation but independent of cell calcium influx [41]. Exosomes are rich in annexins, tetraspanins (CD63, CD81 and CD9) and heat-shock proteins (such as Hsp60, Hsp70 and Hsp90), expose low amounts of phosphatidylserine and include cell-type-specific proteins. In addition, tumor susceptibility gene 101 (Tsg101), Alix and clathrin are frequently expressed in exosomes [42].

Exosomes have been shown to play a key role in immune modulation and cell-to-cell communication [43]. Recent data suggest that the exosomes released from stem cells may deliver bio-active proteins, lipid and nucleic acid cargo to the neighboring diseased or injured cells and bring about the functional changes in the recipient cells [44]. In fact, exosomes produced from a single cell can comprise heterogeneous vesicles [45]. Once released into the extracellular environment, exosomes may interact with recipient cells via adhesion to the cell surface mediated by lipid-ligand receptor interactions, internalization via endocytic uptake or by direct fusion of the vesicles and cell membrane. This may lead to the release of exosome content into the target cell [46]. The outcome of exosome-cell interactions is elicited by modulating the physiology of the target cell, induced through any

of several different mechanisms, ultimately influencing the biology of the tissue/organ. The interactions involve many physiological processes, such as antigen presentation [47], transfer of RNA [48], tissue repair [49], and so forth. Exosomes have also been found to carry antigenic materials and express functional major histocompatibility complexes, giving them the potential to mediate antigen-specific immune responses [50]. The bilayer membrane encapsulation of exosomes provides a protected and controlled internal microenvironment, allowing cargo to travel long distances within tissues without degradation [44]. Most recent developments implicate a major role for exosomes in horizontal transfer of proteins and genetic materials such as messenger RNA (mRNA), micro RNA (miRNA), pre-miRNAs and other non-coding RNAs in the reprogramming of recipient cells [51]. Similarly, it has been reported that the human embryonic and induced pluripotent stem cells derived exosomes chaperone mRNA and proteins [52,53].

Microvesicles

Also known as ectosomes, microparticles shedding vesicles originate from direct budding and blebbing of the plasma membrane of many different cell types [51]. MVs are more heterogeneous in size, ranging from 100 nm to 1000 nm (Table 1), and are released by budding of small cytoplasmic protrusions, which is dependent on calpain, cytoskeleton reorganization and intracellular calcium concentration. Calcium ions are responsible for the changes in asymmetric phospholipid distribution of the plasma membrane that lead to the formation of MVs. These vesicles expose high amounts of phosphatidylserine, contain proteins associated with lipid rafts and are enriched in cholesterol, sphingomyelin and ceramide [54]. These vesicles (MV) contain surface receptors, biologically active molecules such as proteins and lipids, as well as mRNA and microRNA [55].

Apoptotic bodies

Apoptosis is a process that allows an efficient and immunological silent clearing of damaged cells that can be difficult to monitor *in vivo*. Apoptosis is normally characterized by several morphological features, including cell contraction, nuclear condensation, fragmentation and release of apoptotic bodies [56]. Apoptotic bodies, or vesicles, are 1 to 5 μm in diameter (approximately the size range of platelets) and are released from the plasma membrane as blebs when cells undergo apoptosis. They are characterized by phosphatidylserine externalization, which contains several intracellular fragments and cellular organelles, including histones and fragmented DNA [35]. Apoptotic bodies are closed structures, supposed to float on a sucrose gradient at a density between 1.16 and 1.28 g/mL. Their release is dependent on Rho-associated kinase I (ROCK) and myosin ATPase activity, whereas the activity of ROCK proteins is both necessary and sufficient for formation of membrane blebs. However, for re-allocation of fragmented DNA into blebs and apoptotic bodies [57], the role of apoptotic bodies is still not clear. The distinctive features of the three types of extracellular vesicles are summarized in Table 1. MVs may interrelate with target cells by specific receptor-ligand interactions and transfer receptors and biological active molecules to these target cells after internalization [58].

Beneficial effects of MVs/exosomes produced by MSCs

Research to date indicates that stem cell–derived exosomes potentially have significant clinical utility [51]. Exosome-based, cell-free therapies in contrast to cell-based therapies in regenerative medicine can be easier to manufacture and *prima facie* safer, as they are devoid of viable cells and hence there is no risk of tumor formation. Taking advantage of some characteristics of the MSC secretome: (i) they are less immunogenic than parental cells because of lower content of membrane-bound proteins, such as major histocompatibility complex molecules [59]; (ii) exosomes can be stored without potentially toxic cryo-preserved at -20°C for 6 months with no loss of their biochemical activity [60]. An important feature of exosomes is the encapsulation and protection of their contents from degradation *in vivo*, thereby potentially preventing some of the problems associated with small soluble molecules such as cytokines, growth factors, transcription factors and RNAs, which are rapidly degraded [48].

If clinical translation is to be realized, it is important to characterize the physiochemical properties of the purified exosome population along with the constituents of these vesicles in terms of proteins and small RNAs. Therapeutic approaches, based on MSC-derived exosomes and delivery of miRNAs present within them, are currently being explored for some diseases. Figure 3 illustrates the wide array of paracrine molecules released by MSCs with the help of preconditioning factors.

Cardiovascular disease

A recent study has reported that MSCs have the ability to reduce apoptosis by transferring miR-22 in ischemic cardiomyopathy [61]. Additionally, decreased white blood cell count and alleviated inflammation reaction accompanied by decreased infarct size and enhanced cardiac function in hearts after ischemia-reperfusion injury was also shown [62]. These findings reveal that MSC-derived exosomes exert protective effects against cardiovascular diseases through inhibition of inflammation reaction. Another study demonstrated that exosomes derived from MSCs were able to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia-reperfusion injury via activating pro-survival signaling; restoring bioenergetics and reducing oxidative stress [62]. Intact exosomes secreted by MSCs were found to reduce oxidative stress, increase ATP and NADH, control inflammatory activities and activate the PI3K/Akt pathway, leading to protective influences on CMC survival and retention of left ventricular function after ischemia-reperfusion injury. This indicated that exosomes may serve as a supplemental entity for ischemia-reperfusion therapy [62] and also for pulmonary hypertension (PH) by directly suppressing the hypoxic signal pathway and downregulation of proliferative miR-17 superfamily by shifting the balance of proliferation to inhibit PH [63]. Taken together, the above studies suggest that MSC-derived exosomes play an essential role in MSC-based therapy of cardiovascular diseases including myocardial infarction, reperfusion injury and PH [64]. Nevertheless, the detailed mechanisms underlying these benefits of exosomes require further investigation.

Neurological disorders

Because exosomes are lipid-bound nano-vesicles, they act as liposomes and can cross the blood-brain barrier, making them fit for treating neurological disorders. Very recent data

suggest that exosomes could be potentially used as a carrier for brain delivery of anticancer drug for the treatment of brain cancer [65]. It was also proposed that exosomes shuttle miRNAs as regulators for stroke recovery after MSC therapy [66]. On the basis of the potential antioxidant and anti-inflammatory properties of flavonoids, flavonoid-containing exosomes could be used as vehicles to deliver drugs and genetic elements in the treatment of psychiatric and neurologic disorders [67]. When administered to the brain, these exosomes significantly increased myelination and improved re-myelination after injury by prompting pre-oligodendrocytes to differentiate into myelin-producing cells [68].

Kidney damage

Zhou et al. [69] successfully showed that exosomes derived from human umbilical cord mesenchymal stromal cells (hucMSC) can repair cisplatin-induced AKI in rats and NRK-52E cell injury by ameliorating oxidative stress and cell apoptosis, promoting cell proliferation *in vivo* and *in vitro* [69]. It was observed that after administration of cisplatin, there was an increase in blood urea nitrogen and creatinine levels, apoptosis, necrosis of proximal kidney tubules and formation of abundant tubular protein casts and oxidative stress in rats. They showed a significant reduction in all the above indexes with an administration of exosomes from hucMSCs [69].

Immunological diseases

A recent publication revealed that these MSC-derived exosomes serve as vehicles for MSC-specific tolerogenic molecules such as PD-L1, Gal-1, and TGF- β [50]. These observations suggest that MSC-derived exosomes are potent mediators that induce peripheral tolerance and modulate immune responses that provide a new perspective toward indirect application of MSCs in the treatment of autoimmune diseases [70]. MSC-derived exosomes have also been tested in graft-versus-host disease (GVHD) in a recent study. Subcutaneous injection of MSC-derived exosomes in mouse allogeneic skin grafting models delayed the occurrence of GVHD for 2 days [71].

Tumor growth

The extracellular vesicles secreted from MSCs are helpful for transportation of microRNA, proteins and metabolites, which in turn regulate tumor growth [72]. Recent findings suggest that exosomal transfer of miRNAs from the bone marrow may promote breast cancer cell dormancy in a metastatic niche. Acquisition of these dormant phenotypes in bone marrow–metastatic human breast cancer cell line BM2 cells was also observed by culturing the cells in BM-MSC–conditioned medium or with exosomes isolated from BM-MSC cultures, which were taken up by BM2 cells [73]. In another study, MSC-derived exosomes were found to suppress tumor progression and angiogenesis by downregulating the expression of VEGF in tumors *in vitro* and *in vivo*. miR-16, an miRNA enriched in MSC-derived exosomes and known effector of VEGF, was believed to be partially responsible for the anti-angiogenic effect [74].

Bench to bedside

A phase I clinical trial is underway (NCT02138331, 2014) to evaluate the effect of MVs and exosome transplantation on β -cell mass in type I diabetes mellitus (T1DM) (Table 2). The Nassar group hypothesized that intravenous infusion of cell-free umbilical cord–blood derived MSC-MVs may reduce the inflammatory state and hence improve the β -cell mass as well as the glycemic control of the patients of T1DM (Table 2). In another study, the administration of escalating doses of exosomes derived from donor BM-MSCs into a patient with severe therapy-refractory cutaneous and intestinal GVHD grade IV was found to be well tolerated and led to a significant and sustainable decrease of symptoms [75]. These are the only two clinical trials being conducted with stem cell–derived exosomes. A small number of clinical trials are currently investigating dendritic cell (DC)-derived exosomes for immunotherapy or vaccination in severe cancers, such as non-small lung (phase II, NCT01159288) [76], glioma (phase I NCT01550523) [77] and gastric (observational, NCT01779583), and with phase I trials in non-small lung [78], colorectal [79] and melanoma [80]. Interestingly, the use of plant-derived exosomes as vehicles to deliver curcumin to treat colon cancer is also under scrutiny (phase I, NCT01294072) [35].

Side effects of exosomes in therapy

It is imperative to discuss the consequences of exosome therapy to raise a word of caution for its future application. It was recently reported that miRNAs transported by exosomes in body fluids could act as mediators of intercellular communication in cancer [81]. It was also demonstrated that exosomes are associated with an increasing number of neurodegenerative disorders such as Prion, Alzheimer's, Parkinson's disease and Tauopathies [82]. We believe that in-depth research is warranted to gain better understanding on the complexity of exosome cargo and the possible interference of unknown secreted factors.

Summary and future perspective

The translation of therapeutically valuable MSC exosomes into a therapeutic agent presents several unique challenges. One major challenge is to attain current Good Manufacturing Practices (cGMP)-grade exosome production. Desmosomes, a special class of exosomes, were found to be safe in small clinical trials. Unfortunately, lack of large-scale GMP production guidance and several issues such as ethical, legal, technical and regulatory/safety concerns are the challenges. The versatility of MSCs that contributes to restoration of structural integrity and functionality of damaged tissue, including trans-differentiation, cell-fusion, paracrine signaling, exosome secretion and mitochondrial transfer, has drawbacks that must be addressed before maximal benefit is obtained. Trans-differentiation and cell fusion seem to occur in too low a frequency to account for meaningful improvement; exosome secretion and mitochondrial transfer face the problem of finding a robust and scalable cell source with sufficient quantity and quality to generate exosome encapsulation and energy transportation.

With regard to paracrine actions, limitations must not be overlooked. For instance, some cytokines or chemokines released from MSCs may be harmful, such as TNF- α and IL-6. This may explain the modest benefit of MSC transplantation observed in clinical trials [83]. To emphasize the role of surrounding microenvironment and intracellular network to evoke a

better biological response by MSC, we advocate physical, physiological and pharmaceutical preconditioning of MSCs that may help us to achieve a tailor-made secretome profile. MSCs, before collecting secretome, may be subjected to alter external clues, for example hypoxia, treatment with disease-specific drugs, small molecules, specific growth factor/cytokine, use of suitable three-dimensional scaffolds and cellular reprogramming/genetic manipulation, to obtain the suitable secretome. Furthermore, the paracrine action provides the possibility to apply one trophic factor alone or a combination as a cocktail therapy for disease-orientated treatment. Theoretically, the advantage of MSC-based therapy is that it can maintain a sustainable moderate release and concentration of the trophic factors. Thus, harnessing the MSC secretome holds great promise as a controllable, manageable and plausible therapeutic strategy. We envisage that by exploring this angle the clinical translation of stem cell technology might become more feasible in the near future.

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Figure 1.

MSCs can be isolated from various tissues including bone marrow, umbilical cord, muscle and tooth root. After *in vitro* expansion, MSCs can be authenticated as per the guidelines laid down by International Society of Cell Therapy (ISCT). Morphologically, MSCs are fibroblast-like, grow as adherent cultures and are capable of forming colonies; they express a panel of markers: positive for Sca-1, CD105, CD73, CD29 and CD90 and negative for CD31, CD34, CD45 and CD11b. In addition, MSCs have the potential to differentiate into adipocytes, chondrocytes, osteoblasts and other cell types.

Figure 2.

Schematic representation depicting the origin of MVs, exosomes and apoptotic bodies. An MV arises from budding of the plasma membrane. MVs are more irregular in shape and size and can contain cytoplasmic materials; they express surface markers such as integrin- β , CD40 and selectins such as plasma selectins and/or proteins similar to the cells from which they originate. Exosomes are derived from the endosomal trafficking system and therefore are more regular in shape and size. Exosomes are more easily identifiable via cell surface markers such as CD81, CD9 and CD63 and may contain materials such as mitochondrial DNAs, mRNAs and miRNAs. ER, endoplasmic reticulum; miRNA, microRNA; TGN, trans-

Golgi network; MVB, multi-vesicular bodies. Apoptotic bodies or vesicles are 1 to 5 μm ($>1000\text{ nm}$) in diameter; they are released from the plasma membrane as blebs when cells undergo apoptosis and contain several intracellular fragments and cellular organelles.

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Figure 3.

Role of various paracrine factors released by MSCs. Secreted factors may exert different functions on cells via the release of different kinds of molecules, depending on the microenvironment: angiopoietin (Ang), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), chemokine ligand (CCL), chemokine (C-XC motif) ligand (CXCL), erythropoietin (EPO), glial cell line–neurotrophic factor (GDNF), granulocyte-macrophage–colony-stimulating factor (GM-CSF), hemeoxygenase (HO), hepatocyte growth factor (HGF), human leucocyte antigen (HLA), indoleamine 2,3-dioxygenase (IDO),

insulin growth factor (IGF), interleukin (IL), keratinocyte growth factor (KGF), leukemia inhibitory factor (LIF), human cathelicidin (LL37), monocyte chemoattractant protein (MCP), metalloproteinase (MMP), nerve growth factor (NGF), nitric oxide (NO), platelet-derived growth factor (PDGF), prostaglandin (PGE), placental growth factor (PIGF), stem cell factor (SCF), stromal cell–derived factor (SDF), tissue inhibitor of metalloproteinase (TIMP), transforming growth factor (TGF), thrombopoietin (TPO), TNF- α –stimulated gene/protein (TSG) and vascular endothelial growth factor (VEGF).

Table 1

Key features of exosomes, microvesicles and apoptotic bodies.

	Exosomes	Microvesicles	Apoptotic bodies
Size (nm)	40 to 100	100 to 1000	>1000
Genesis	By exocytosis of multivesicular bodies. Process dependent on cytoskeleton activation and Ca^{2+} -independent.	By budding of plasma membranes. Process dependent on Ca^{2+} , calpain and cytoskeleton reorganization	By blebbing of plasma membranes of dying cells.
Identification	CD63, CD81, CD82, CD9, Tsg101, Alix, HSP70 and HSP 90. Markers specific to the cell of origin, eg, PECAM, in platelet vesicles and EGFRvIII in vesicles from gliomas	Lipid raft-associated molecules (TF, flotillin)	Exposure of PS
Content	Proteins, lipids, mRNA and microRNA, rarely DNA	Proteins, lipids, mRNA and microRNA, rarely DNA	Fragmented DNA
Isolation technique	Centrifugation at 100,000 <i>g</i> followed by 2 h Electron microscopy	Ultracentrifugation Electron microscopy	Flow cytometry Electron microscopy
Storage	−20°C	−20°C	N/A

Table 2

Cell secretome (exosome)-based clinical trials.

Country	Sponsor	Title (interventions/indication)	Trial stage	ClinicalTrials.gov Identifier (estimated enrollment)
Egypt	General Committee of Teaching Hospitals and Institutes	Effect of Microvesicles and Exosomes Therapy on β -cell Mass in Type I Diabetes Mellitus (T1DM)	Phase 1	NCT02138331 (20)
France	Gustave Roussy	Trial of a Vaccination With Tumor Antigen-loaded Dendritic Cell-derived Exosomes	Phase 2	NCT01159288 (47)
Spain	Hospital Miguel Servet	Circulating Exosomes As Potential Prognostic And Predictive Biomarkers In Advanced Gastric Cancer Patients ("EXO-PPP Study")	Observational	NCT01779583 (80)
United States	Thomas Jefferson University	Pilot Immunotherapy Trial for Recurrent Malignant Gliomas	Phase 1	NCT01550523 (12)
United States	James Graham Brown Cancer Center	Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Phase 1	NCT01294072 (35)