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Extracellular vesicles derived from Mesenchymal Stem/Stromal cells: Emerging therapeutic perspectives

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Mesenchymal Stem/Stromal Cells (MSCs) are multipotent stem cells capable of differentiating into various mesodermal lineages, including adipose, bone, cartilage, muscle and tendon [1]. They are found in various human tissues such as bone marrow, umbilical cord blood, adipose tissue, lung parenchyma, placenta, peripheral blood and even dental pulp [1]. MSCs constitute one of the principal components of tissue microenvironment, exhibiting a key role in maintaining homeostasis [2].

Because of the ease of isolation, plasticity, homing to injured tissues and their immunosuppressive and immunomodulatory properties, MSCs have emerged as appealing candidates for various therapeutic applications including tissue repair and regeneration, treatment of autoimmune disorders and cancer [3]. There is a large body of evidence to suggest that MSCs exert their therapeutic effects mostly by the release of various agents rather than through cell to cell interactions [4]. Their secretome is rich of growth factors, cytokines, chemokines and extracellular vesicles (EVs) [5]. The latter are small membrane-coated particles secreted by cells containing mRNA, miRNA, DNA, proteins and lipids [4]. EVs have been shown to play a pivotal role in cell-to-cell crosstalk, mediating both local and distant communication [4]. EVs can be classified into three major subtypes based on their size and biogenesis: apoptotic bodies, microvesicles and exosomes [4]. Apoptotic bodies, with a diameter ranging from 50nm to 2 μm, are produced during programmed cell-death, via the plasma membrane blebbing. Microvesicles (MVs) with a size ranging from 150 nm to 1μm are formed through the outward budding of the cell membrane. Finally, exosomes, which are the smallest nanoparticles with a diameter from 40 to 150 nm originate from the invagination of the endosomal membrane to form multi-vesicular-bodies, which then fuse with the cell membrane, thereby resulting to the secretion of exosomes [4]. However, as clearly stated in a recent position paper of the International Society for Extracellular Vesicles, the distinction of the aforementioned EV-subtypes remains challenging [6].

Several methods have been implemented for the isolation of EVs based on their size or protein cargo, including differential centrifugation, filtration, chromatography and immunoaffinity-based technics [5]. Furthermore, various assays have also been proposed for the characterization and quantification of EVs, based either on their physical properties such as dynamic light scattering, flow cytometry, electron microscopy, nanoparticle tracking analysis and tunable resistive pulse-sensing or on their biochemical properties including immunoblotting, immuno-sorbent analysis, ELISA and total protein colorimetric assays [5]. Notably, there is no single optimal EV isolation, characterization or quantification method [5].

The application of MSCs in cell-based therapies reflects their potential to migrate, engraft and interact with other cells, especially in inflamed or damaged tis-

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sues [7]. However, despite the well documented efficacy of MSC therapy in both preclinical and clinical studies, this therapeutic modality has raised concerns regarding potential infusion toxicities, undesired differentiation, genetic instability of the ex vivo expanded cells and tumor formation risk [8]. On the other hand, a rapidly growing body of evidence has clearly demonstrated that MSC-derived EVs retain the biological activity and therapeutic potential of MSCs and can therefore be considered as an alternative cell-free therapeutic approach [8]. Interestingly, EVs may be more attractive that their cellular counterparts due to their favorable safety profile. In fact, they are less immunogenic than MSCs, they are non-replicative and their use bypasses the transfer of cells harboring potentially mutated or damaged DNA. In addition, due to their small size MSC-derived EVs can readily circulate in contrast to MSC which are larger and are often trapped in lung capillary beds, an issue that eventually hampers their systemic administration [5].

In order to achieve an enhanced therapeutic effect, EVs can be modified and loaded with molecules of interest. To this end, parental MSCs can be manipulated in order to produce EVs carrying specific cargoes. Alternatively, naive EVs can be processed and loaded exogenously [4]. In this way, RNA molecules or proteins with therapeutic potential can be packed in EVs and delivered to specific recipient cells thereby providing a targeted treatment approach [4].

The clinical benefits of MSC-EVs as regards to tissue repair, immune modulation and microenvironment crosstalk have been investigated in various disease settings. Thus far, encouraging results have been reported in several animal models and human studies [7].

Within the context of cardiovascular regeneration, MSC-EVs' administration has been shown to mitigate ischemia-reperfusion injury via the modulation of Akt and MAPK8 pathways in mice [9]. Furthermore, through the up-regulation of myocardial LC3B, an autophagy related protein, MSC-EVs reduced infarct size and improved heart function in myocardial ischemia rat models [10]. Additionally, in vivo angiogenesis and blood reperfusion was enhanced by MSC-EVs in a murine limb ischemia experimental setting [11].

Several studies have demonstrated the potential efficacy of MSCs-EVs in the treatment of acute respiratory distress syndrome (ARDS), as reviewed by Shah et al [12]. To this end, administration of MSC-derived EVs enhanced anti-inflammatory cytokine production, decreased apoptosis and reduced inflammatory cells influx in murine models. In addition, MSC-derived EVs exhibited regenerative features as they restored endothelial cells' tight junctions, thereby reducing protein permeability and pulmonary edema [12]. An anti-inflammatory pattern was also demonstrated in a hypoxia-induced pulmonary hypertension model by using umbilical cord MSC- EVs [13]. Furthermore, in an animal model of lower respiratory viral infection, the intratracheal administration of MSC-EVs had beneficial effects, as evidenced by the inhibition of influenza virus shedding and replication as well by the reduction of inflammatory lung lesions [14]. Recently, the therapeutic role of MSCs has been addressed in COVID-19 patients. Results are promising, as suggested by the clinical improvement being attributed to MSC anti-inflammatory effect [15]. In an attempt to overcome the aforementioned limitations of cellular therapy, a clinical study using MSC derived exosomes in patients with SARS-CoV2 infection has been designed (identification No. NCT04276987).

The regenerative potential of MSC-EVs has also been demonstrated in acute kidney injury animal models. In that experimental setting MSC-EVs were effective in improving renal function, decreasing fibrosis and lymphocyte infiltration as well as accelerating the proliferation of tubular cells [4]. Human trials have shown promising results as well. In a phase II/III clinical trial, the administration of MSC-EVs in patients with chronic kidney disease led to improvement of eGFR and decreased albuminuria [16]. This beneficial effect is probably attributed to the modulation of chronic inflammation, as the levels of anti-inflammatory cytokines TGF-β and IL-10 were increased while those of the pro-inflammatory cytokine TNF-α were decreased in patients treated with MSV-EVs [16].

The therapeutic efficacy of MSC-EVs has also been investigated in liver disorders. More precisely, in murine models of acute hepatic failure and liver fibrosis MSC-EVs were able to regulate inflammatory cytokine pathways, reduce liver injury and increase survival [17].

Moreover, in a mouse model of type 1 diabetes, MSC-EVs suppressed Th1 and Th17 response due to their immunomodulatory potential [18]. Based on these findings, a clinical trial evaluating the use of MSC-EVs in patients with type 1 diabetes mellitus has been undertaken (identification No. NCT02138331). The results of this trial are eagerly awaited.

Finally, as exosomes have been shown to bypass the blood-brain barrier, their role in central nervous

system disorders is drawing much attention and this is supported by encouraging preclinical and clinical data. For example, MSC-EVs loaded with miR-124 promoted post-stroke neurovascular recovery in murine models [19]. Based on this, a phase I/II clinical trial is aiming to assess allogenic miR-124 bearing exosomes' regenerative effect after acute ischemic stroke (identification No. NCT03384433).

However, despite the aforementioned studies reporting encouraging results regarding the therapeutic potential of MSC-EVs, there are still important obstacles to overcome so as to optimize their clinical use. To this end, standard validated protocols for the isolation, large scale preparation, characterization and storage of EVs have to be established, along with clearly defined quality control (QC) criteria for cellular therapeutics [7]. This is expected to diminish the heterogeneity of EV batches, which currently results in unpredictable therapeutic efficacy, as documented in some clinical trials. Finally, despite the fact that the few existing preclinical studies have not reported toxicities or harmful effects of MSC-EVs, clinical trials are absolutely required so as to establish a safety profile and determine the optimal dosage before cell-free therapies find their way to the clinics.

In conclusion, due to their size, ability to transport genetic material and potential to mediate immunosuppressive and other MSC paracrine-acting effects, MSC-EVs represent a promising treatment modality in various areas of medicine including inflammatory disorders, regenerative medicine and cancer. Additional research is warranted though, in order to extend existing knowledge on MSC-EVs and pave their way for clinical applications.

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