Journal of Advanced Research 70 (2025) 103–124

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

Review Article

Mesenchymal stem cell secretome for regenerative medicine: Where do we stand?

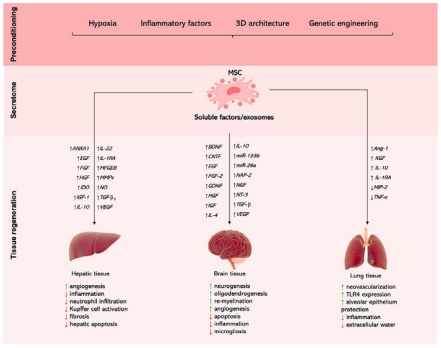
Catarina M. Trigo, Joana S. Rodrigues, Sérgio P. Camões, Susana Solá [1], Joana P. Miranda *,[1]

Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

HIGHLIGHTS

- MSCs empower the resident cells, to regenerate the damaged tissue, via secretion of trophic factors.
- MSC secretome contains immunomodulatory factors regulating innate and adaptive immune responses.
- The content profile of the MSC secretome is "personalized" according to local microenvironmental cues.
- Engineering strategies can be adopted to modulate/boost the therapeutic features of MSC secretome.
- MSC secretome has proved therapeutic potential in respiratory, liver, and neurological diseases.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 August 2023
Revised 27 February 2024
Accepted 3 May 2024
Available online 9 May 2024

Keywords:
Extracellular vesicles
Mesenchymal stem cells
Preconditioning strategies
Regenerative medicine
Secretome

ABSTRACT

Background: Mesenchymal stem cell (MSC)-based therapies have yielded beneficial effects in a broad range of preclinical models and clinical trials for human diseases. In the context of MSC transplantation, it is widely recognized that the main mechanism for the regenerative potential of MSCs is not their differentiation, with *in vivo* data revealing transient and low engraftment rates. Instead, MSCs therapeutic effects are mainly attributed to its secretome, i.e., paracrine factors secreted by these cells, further offering a more attractive and innovative approach due to the effectiveness and safety of a cell-free product.
Aim of review: In this review, we will discuss the potential benefits of MSC-derived secretome in regenerative medicine with particular focus on respiratory, hepatic, and neurological diseases. Both free and vesicular factors of MSC secretome will be detailed. We will also address novel potential strategies capable of improving their healing potential, namely by delivering important regenerative molecules according to specific diseases and tissue needs, as well as non-clinical and clinical studies that allow us to dissect their mechanisms of action.

Abbreviations: 6-OHDA, 6-hydroxydopamine; APC, antigen presenting cell; AT-MSCs, adipose tissue-derived mesenchymal stem cells; BALF, bronchoalveolar lavage fluid; BBB, blood–brain barrier; BM, bone marrow; BMMNCs, bone marrow mononuclear cells; CM, conditioned media; CM2D, two-dimensional conditioned media; CM3D, three-dimensional conditioned media; CNS, central nervous system; COVID-19, coronavirus disease 2019; CTLs, T cytotoxic lymphocytes; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; ECM, extracellular matrix; EVs, extracellular vesicles; GMPs, good manufacturing practices; HSCs, hepatic stellate cells; hUC, human umbilical cord; iNOS, inducible nitric oxide synthase; MMPs, matrix metalloproteinases; MS, multiple sclerosis; MSCs, mesenchymal stem cells; NAFLD, non-alcoholic fatty liver disease; NK, natural killer; NPCs, neural progenitor stem cells; NSCs, neural stem cells; SCI, spinal cord injury; Th1, T helper 1 cells; Th17, T helper cells 17; TLR-4, toll-like receptor 4; Tregs, T regulatory cells; UC-MSCs, umbilical cord-derived mesenchymal stem cells.

* Corresponding author at: Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal.
E-mail address: jmiranda@ff.ulisboa.pt (J.P. Miranda).

[1] Equally contributed as senior authors.

https://doi.org/10.1016/j.jare.2024.05.004

Key scientific concepts of review: MSC-derived secretome includes both soluble and non-soluble factors, organized in extracellular vesicles (EVs). Importantly, besides depending on the cell origin, the characteristics and therapeutic potential of MSC secretome is deeply influenced by external stimuli, highlighting the possibility of optimizing their characteristics through preconditioning approaches. Nevertheless, the clarity around their mechanisms of action remains ambiguous, whereas the need for standardized procedures for the successful translation of those products to the clinics urges.

Contents

Introduction . . . 104
Secretome of MSCs . . . 104
Immunomodulatory properties of MSC secretome . . . 105
Innate immunity response by MSC secretome . . . 105
Adaptive immunity response by MSC secretome . . . 106
Angiogenic properties of MSC secretome . . . 107
Anti-tumoral properties of MSC secretome . . . 107
Current trends to ameliorate MSC secretome . . . 108
Hypoxia-preconditioned MSCs . . . 108
Inflammatory-preconditioned MSCs . . . 109
3D culture preconditioned MSCs . . . 109
Genetic-engineered MSCs . . . 110
MSC secretome in regenerative medicine . . . 110
MSC secretome in respiratory diseases . . . 110
MSC secretome in liver diseases . . . 111
MSC secretome in neurological diseases . . . 113
Conclusions and future perspectives . . . 115
Compliance with ethics requirements . . . 117
Declaration of competing interest . . . 117
Acknowledgments . . . 118
References . . . 118

Introduction

It has been half a century since Alexander Friedenstein first reported mesenchymal stem/stromal cells (MSCs) as clonogenic progenitor cells able to give rise to fibroblasts and other mesodermal cells [1], being later identified with great potential for regenerative medicine application. At first, it was postulated that, upon administration, MSCs would migrate into injured sites, engraft, and further differentiate into end-stage functional cells, thus repairing the damaged tissue [2,3]. However, decades have passed, and many reports based on animal studies or even in human clinical trials have questioned this theory. MSCs have shown their therapeutic effects in numerous disease models as well as in some clinical trials [4]. It turned out to be more evident that the rate and duration of MSC engraftment were too limited to justify the remarkable results observed in tissue repair. Therefore, a new perspective began to take shape with MSCs derived from bone marrow (BM)-MSCs being able to ensure the growth and viability of hematopoietic stem cells in co-culture conditions without growth factor supplementation [5,6]. These initial observations extended the MSC functionality beyond their capacities for engraftment and differentiation. MSCs were shown to empower the resident cells through the secretion of trophic factors, inducing those cells to regenerate the damaged tissue [7–10]. In fact, it has been proposed that upon tissue damage, endogenous MSCs migrate from their perivascular location and create a regenerative microenvironment through the secretion of bioactive factors and the modulation of the immune cell response [11,12]. Nowadays, it is well recognized that MSC exert their therapeutic effects primarily through paracrine signaling and that these cells could adapt their paracrine action according to local microenvironmental cues [13]. This characteristic of MSCs has been the one currently granting the most clinical interest which culminates in the use or/and modulation of the MSC-derived secretome (or conditioned media – CM), with multiple studies being performed for different types of disorders and medical conditions. Of note, some studies involve the use of the cells secretome or the administration of the cells itself, for the evaluation of their paracrine effect [14]. Nevertheless, clinical trials involving the whole MSC-derived secretome have already been started for osteoarthritis [15], ischemic stroke and other cerebrovascular conditions [16–18], neurological pathologies, such as Alzheimeŕs disease [19], respiratory diseases [20–22], including severe cases of coronavirus disease 19 (COVID-19) [23–31], among others. This review highlights how MSC-derived secretome can be optimized/potentiated and thus explored for applying in respiratory, liver, and neurological disorders.

Secretome of MSCs

MSCs are stromal nonhematopoietic cells with spindle shape [32], highly replicative *in vitro*, that derive from the mesoderm germ layer from different tissues [33–35]. The minimal guidelines to define human MSC identity have been described by the International Society for Cellular Therapy (ISCT) and define that the isolated cells are generally positive for CD105, CD73, and CD90, and negative for CD45, CD34, CD14, or CD11b, CD79α, or CD19 and MHC class II [36]. Based on their origin, MSCs can be classified into *i)* neonatal tissues, such as placenta, fetal blood, umbilical cord tis-

Table 1
MSC-released factors with immunomodulation properties.
Abbreviations: BLM: bleomycin; BM: bone marrow; CLP: cecal ligation and puncture; EAE: experimental autoimmune encephalomyelitis; EVs: extracellular vesicles; GvHD: graft vs. host disease; HGF: hepatocyte growth factor; hMSCs: human mesenchymal stem cells; hPDLSCs: human periodontal ligament stem cells; IDO: indoleamine-pyrrole 2,3-dioxygenase; IFN-γ: interferon-gamma; IL-1RA: interleukin 1 receptor antagonist; IL: interleukin; MI: myocardial infarction; miRNA: micro-RNA; MS: multiple sclerosis; MSCs: mesenchymal stem cells; PGE_2: prostaglandin E_2; TLR4: toll-like receptor 4; TNF-α: tumor necrosis factor; TSG-6: anti-inflammatory; TNF-α-stimulated gene 6 protein.

Paracrine factor	Disorder	Summarized of beneficial outcome	Type of MSCs	Animal model	Ref.
HGF	MS	– Enhances neuroregeneration and synaptogenesis; – Cooperates on axonal growth; – Reduces pro-inflammatory cytokines released by mononuclear cells (IFN-γ, IL-17, TNF-α, IL-2, IL-12p70) and increases the anti-inflammatory mediators IL-4 and IL-10.	BM-MSCs	EAE animal model	[236,260]
IL-10	MS	– IL-10 release is characteristically decreased prior to relapse phases and increased during remission phases of MS; – IL-10-deficient animals usually develop a more severe disease.	hPDLSCs-derived EVs	EAE animal model	[69],261]
IL-1RA	Acute lung injury	– MSC-CM blocked an IL-1α-dependent T cell line. This effect was abrogated using an IL-1RA antibody. – MSC administration significantly decrease IL-1α mRNA in lung 7 and 14 days after exposure to BLM.	Murine-MSCs and hBM-MSCs	BLM-induced lung injury	[175]
PGE_2	Sepsis	– Endotoxins present in sepsis stimulates TLR4 present in MSCs, enhancing their production of PGE_2; – The release of PGE_2 exerts anti-inflammatory effects, inducing the switch of macrophages into M2 state.	BM-MSCs	CLP animal model	[70,73,262]
TSG-6	MI	– TSG-6 inhibits neutrophils infiltration into sites of inflammation; – The role of TSG-6 on cardiac function is not so well-established but there are studies that point in this direction.	BM-MSCs	MI animal model	[62]
IDO	GvHD	– IDO suppresses T cell responses; – IDO is associated with the regulation of autoimmune conditions, inducing donor tolerance.	BM-MSCs	Kidney allograft animal model	[62,263,264]

sue (Wharton's jelly) and cord blood; or *ii)* adult tissues, including bone marrow, thymus, brain, liver, lung, kidney, aorta, muscle, spleen and adipose tissue (AT) [37,38]. Although MSCs of all different sources must fulfil the defined criteria defined by the ISCT, they diverge in proliferation and growth rates, differentiation and immunomodulatory potentials and regenerative properties [38]. Moreover, MSC-derived secretome itself may present different contents depending on the tissue of origin, cell culture conditions or cellular microenvironment [39–41], turning hard the identification of a precise mechanism of action. Nevertheless, the anti-inflammatory and immunomodulatory capacities of MSCs (Table 1) were postulated as essential to retrieve local and systemic conditions for normal tissue repair alongside MSC-mediated pro-angiogenic, anti-apoptotic and antimicrobial effects (Table 2) [8,9,12,39,42,43].

The cell secretome, consists of soluble (growth factors, cytokines, chemokines, and hormones) factors and of non-soluble factors contained in extracellular vesicles (EVs). Nowadays, according to Minimal Information for Extracellular Vesicles 2018 (MISEV 2018), EVs (sEVs) are classified in small EVs or medium/large EVs (m/lEVs) if they have <100 nm or <200 nm (small) or >200 nm, respectively [36]. The exosomes, in particular, are EVs within the sEVs, as the apoptotic bodies, therefore, exosomes classification further includes demonstration of function (e.g. internalization, effect, origin from the endosomal system). In what concerns to the MSC-EVs mediated effects, it is related to their cargoes, including proteins, functional mRNA, miRNAs and lipids [44–46]. Specifically, the miRNAs present in sEVs can regulate between 30 % and 70 % of the gene expression [46]. Moreover, many miRNAs have been related with regenerative functions in different organs. For example, miR-199a is being related with cardiomyocyte proliferation, miR-23-a-p and miR-130-a-3p with angiogenesis and vascular development [46], mi-R-126-3p, miR-223-3p and miR-142-3p with immunomodulatory effects [47], miR-133b [48], miR-26a [49] and miR-124 [50] with protective properties in the neurological and cerebrovascular field. Nevertheless, despite some evidence that MSC-EVs have an important role in mediating the regenerative and immunomodulatory properties of MSCs [51,52], a completely consensus is not established [53,54], with some data showing that the total MSC-secretome and MSC-EV-free fraction had better immunomodulatory effects when compared with MSC-EVs [55–57].

For all of this, the interest of MSC-derived secretome is notably increasing, specially the secretome-derived EVs for its easily mode of preservation and transference [58] as well as their biocompatibility. The clinical administration of EVs is also more convenient, reproducible and predictable than MSCs [56], although the exact mechanism of the uptake of these vesicles through the biological membranes occurs remains a matter of debate [59].

Immunomodulatory properties of MSC secretome

Innate immunity response by MSC secretome

Many soluble factors and EVs released by MSCs are known to modulate immune responses via cell-to-cell contact and by an ample number of cytokines and regulatory factors that influence the recruitment, proliferation, activation, function, and survival of several immune cells (Table 1) [60,61].

MSCs have shown dual activity regarding neutrophil recruitment *vs.* inhibition. Some evidence has suggested that by releasing IL-6 [42], TSG-6 [62] and superoxide dismutase (SOD3) [63], BM-MSCs can reduce apoptosis of neutrophils that are resting or activated [42] (Table 1). Moreover, MSCs also secrete CXCL8 (also known as IL-8) and macrophage migration inhibitory factor (MIF), known for recruiting neutrophils into the injured site [42,64]. The release of these molecules is considered a relevant mechanism to preserve the pool of neutrophils and contribute to the resolution of the infection. In contrast, other authors state that MSCs inhibit neutrophils activity [65] (Fig. 1). For example, TSG-6, an anti-inflammatory protein considered a key factor in immunomodulatory activity of MSCs [66], was shown to inhibit neutrophils migration to the injured site possibly by binding to IL-8 and, as such, to inhibit the neutrophils recruitment by this chemokine [67]. Further, in a model of endotoxin-induced lung injury, the MSC-derived-EVs inhibited the migration of neutrophils into the lung [42,68] while IL-10 released by MSCs appeared to limit the pro-inflammatory state of polymorphonuclear neutrophils [69].

Table 2
Application of MSC secretome in bone tissue, cardiovascular, intestinal, and renal diseases.
Abbreviations: Arg-1: arginase-1; AKT: protein kinase B (PKB)/serine/threonine kinase; Bcl-2: B-cell lymphoma 2; bFGF: basic fibroblast growth factor; FGF: fibroblast growth factor; GFR: glomerular filtration rate; IGF-1:insuline-like growth factor 1; IL-3/-6/-10: interleukin 3/6/10; HGF: hepatocyte growth factor; MCP-1/-3: monocyte chemotactic protein-1; MSCs: mesenchymal stem cells; VEGF: vascular endothelial growth factor; MAPK/ERK1/2: mitogen-activated protein kinase/ extracellular signal-regulated protein kinase ½; TB4: thymosin beta-4; TGF-α: transforming growth factor alpha.

Application	Main effects	References
Cardiovascular diseases	• Cytoprotection and regenerative effects on ischemic cardiomyocytes through the secretion of VEGF, HGF, FGF, IGF-1, and TB4. • Anti-fibrotic effects through the inhibition of type I and type III collagen expression in cardiac fibroblasts. • Acceleration of re-endothelialization through VEGF activation via AKT and MAPK/ERK1/2 pathways. • Alleviation of vascular calcification by reducing intracellular calcium content and alkaline phosphatase activity.	[160–164]
Bone tissue diseases	• Bone regeneration in calvarial bone defects, possibly through the secretion of VEGF and IGF-1. • Angiogenesis, bone regeneration and potential to mobilize endogenous MSCs in a rat calvarial defect model via VEGF. • Accelerated callus formation in the setting of distraction osteogenesis through the recruitment of endogenous MSCs, induction of osteoblast differentiation, angiogenesis, and cell proliferation and inhibition of inflammation and apoptosis via MCP-1/-3 and IL-3/-6.	[165–167]
Renal diseases	• Improved renal function, higher proliferative and lower apoptotic indexes with increases in the expression of bFGF, IL-10, TGF-α and Bcl-2. • Decreased renal deterioration in a chronic kidney disease model, with higher GFR and effective renal plasma flow and lower systolic blood pressure, proteinuria and tubular and glomerular damage. • Polarization of macrophages into M2 anti-inflammatory phenotype with upregulation of Arg-1 expression, via MSC secretion of IL-6.	[168–170]
Intestinal diseases	• Decreased pro-inflammatory response by downregulation of genes coding for pro-inflammatory markers IL-1β, IL-6, TNF-α, and TLR4 and by upregulation of the anti-inflammatory IL-10. • Improved migratory effect on lymphatic endothelial cells along with the alteration of secretome protein levels upon VEGF stimulation of MSCs (reduced levels of the pro-inflammatory cytokines IL-6 and IFN-γ and higher concentrations of TGF-β1, FGF-2 and VEGF-C).	[158,159]

Interestingly, macrophages can be reprogrammed to increase IL-10 to attenuate sepsis. This was observed in mouse sepsis models, through PGE_2 secretion of TNF-α-primed BM-MSCs [70]. Indeed, MSCs were shown to polarize macrophages from an inflammatory M1 phenotype to an anti-inflammatory M2 phenotype [4]. The MSC-induced M2 phenotype polarization results in an increased levels of arginase-1 (Arg-1) and of the anti-inflammatory cytokines IL-10 and TGF-β1. Moreover, it has been associated to a reduced production of pro-inflammatory cytokines, such as IL-1β, IL-12, IL-23 and TNF-α and of the co-stimulatory molecules CD86 and MHCII [71,72] (Table 1). Importantly, if, on one hand, factors segregated by MSCs such as IL-1RA [73,74] inhibit the product of TNF-α segregated by IL-1α-activated macrophages [73], on the other hand, inflammatory factors segregated by macrophages, such as TNF-α [4,63] and LPS [63] activate MSCs to segregate anti-inflammatory mediators, including TSG-6 [4,63], IL-10 [4,63] and PGE_2 [4,63] (Fig. 1). Notably, Hyvarinen and co-workers have shown that BM-MSCs co-culture with regulatory macrophages and MSC-EVs decrease the levels of IL-22 and IL-23 in the macrophages-produced CM [75]. The authors also demonstrated that MSC-EVs mediate their action, at least in part, via PGE_2 (Table 1) [75].

On the other hand, it has been also demonstrated that MSCs can disrupt major functions of dendritic cells (DCs), namely their migratory ability in response to CCL19 and antigen presentation to T cells. DCs are maintained in an immature or semimature state due to the release of PGE_2, HLA-G and IL-10 (Fig. 1). In fact, the secretion of IL-10 is thought to affect the JAK1/STAT3 signaling pathway, as well as through the release of TNF-α, suppressing the MAPK and NF-κB signaling activation [63,76], signaling pathways which are involved in the production of pro-inflammatory modulators [77,78].

Curiously, IL-1RA, a natural-occurring antagonist of IL-1α and IL-1β signaling pathway present in MSC-secretome, has also demonstrated to suppress IL-1β:IL-1R/ NF-κB signaling pathway, reducing the production of IL-1β, IL-6, IL-12 and IL-23 in M1 macrophages and DCs and attenuating the expansion of T helper 1 cells (Th1) and T helper cells 17 (Th17) [74,79] (Fig. 1, Table 1). It was also suggested that some miRNAs present in MSC-derived EVs are capable of suppressing DC activity, such as mi-R-126-3p, miR-223-3p and miR-142-3p [47]. MSCs can also induce mature DCs to differentiate into regulatory DCs. However, the mechanism and the main released factors responsible for this immunomodulation effect need further investigation [80]. These suppressive effects of MSCs on DCs are of great relevance for many immune situations, including GvHD [81] and allograft rejection [82].

Finally, although substantial data suggests that both cell types interact and affect each other, the crosstalk between natural killer (NK) and MSCs is still an area in its infancy. There are still conflicting studies reporting the MSCs inhibitory/stimulating effect in NK. Besides the fact that modulation effect was higher in direct cell-to-cell contact, soluble factors serve as an additive factor that turns MSCs more powerful in altering the cell function of NK [63,83]. PGE_2 [84], TGF-β [83] and the immunosuppressive enzyme IDO [85] secretion by MSCs impaired the proliferation and cytotoxicity of NK cells, while activin A showed to be able to suppress IFN-γ-production by NK cells [84]. In addition, the ratio between NK and MSCs was found to be important for the MSC-immunosuppressive-mediated effects [86].

Adaptive immunity response by MSC secretome

MSCs are also known to suppress the proliferation and activation of T cells, namely of $CD4^+$ T cells (Th) and $CD8^+$ T cells (T cytotoxic lymphocytes, CTLs) [87,88]. Indeed, umbilical cord-derived

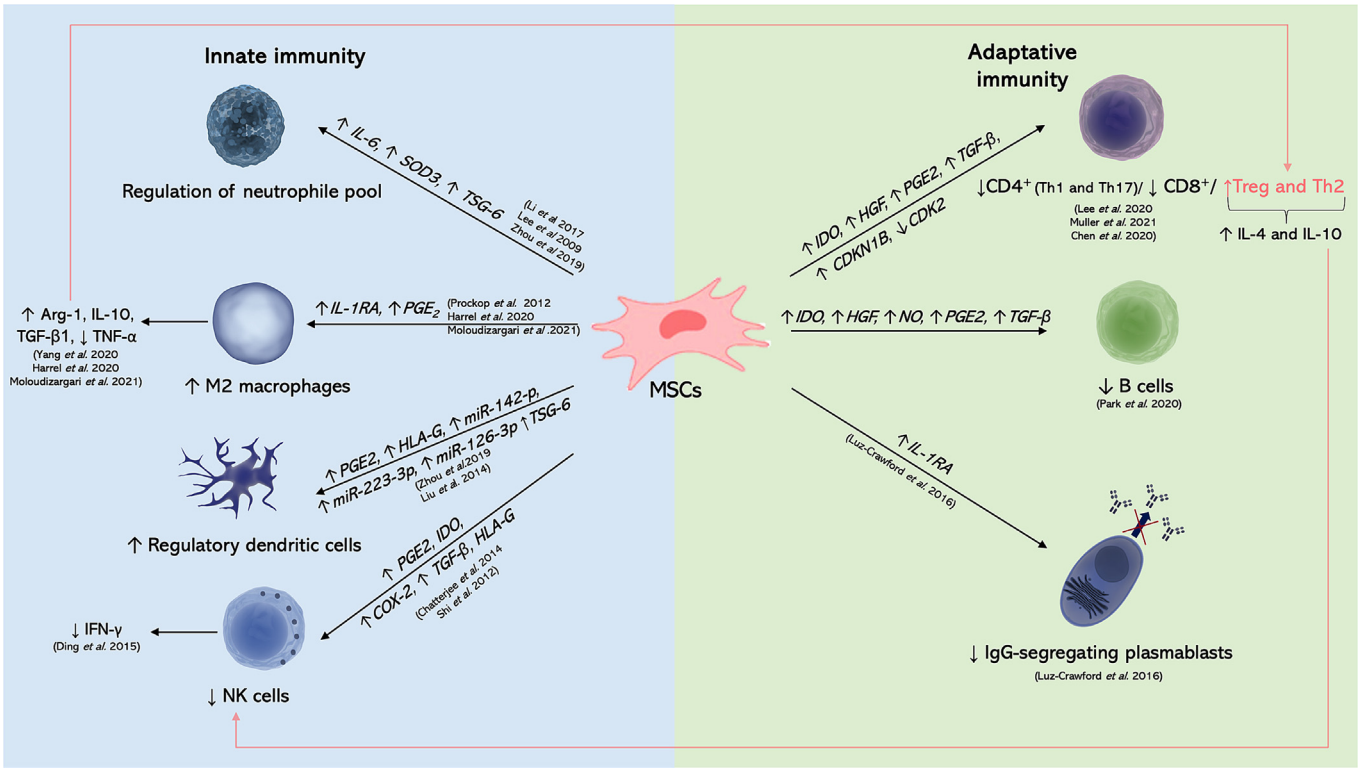


Fig. 1. MSCs exert several immunomodulatory effects by regulating both innate and adaptive immune cell populations.
Abreviations: Arg-1: arginase-1; CTLs; cytotoxic T lymphocytes; CDKN1B: cyclin dependent kinase inhibitor 1B; CDK2: cyclin-dependent kinase 2; COX-2: cyclooxygenase 2; HLA-G: major histocompatibiliy complex, class I, G; HGF: hepatocyte growth factor; IDO: indoleamine 2,3-dioxygenase; IFN-γ: interferon-gamma; IL: interleukin; IL-1RA: interleukin 1 receptor antagonist; LPS: lipopolysaccharide; miR: miRNA; GM-CSF: granulocyte-macrophage colony-stimulating growth factor; NK: natural killer; NO: nitric oxide; PGE2: prostaglandin E2; SOD3: extracellular superoxide dismutase; TGF-β: transforming growth factor beta; Th1: T helper cells 1; Th2: T helper cells 2; Th17: T helper cells 17; TNF-α: tumor necrosis factor alpha; TSG-6: anti-inflammatory TNF-α-stimulated gene 6 protein.

mesenchymal stem cells (UC-MSCs) induced relevant T cell apoptosis and cell arrest through abundant expression of IDO [89]; whereas IDO has been also shown to enhance the expansion of $CD4^+$ $CD25^+$ T regulatory cells (Tregs) [8] (Fig. 1 and Table 1). Apart from the apoptotic and anti-proliferation effects, MSCs seem to inhibit the generation and function of Th1 and Th17 cells whilst promoting T helper cells 2 (Th2) cells and Tregs, resulting in a decrease of pro-inflammatory cytokines and an increase of IL-4 and IL-10 [88].

Moreover, B cell proliferation has been shown to be inhibited by MSCs through an arrest in the G_0/G_1 phase of the cell cycle. It was reported that the secretion of IL-1RA or TGF-β by human cord blood-derived mesenchymal stem cells (hCB-MSCs) impaired the maturation of B cells [90,91] (Fig. 1). Similarly, Magatti *et al.* reported that culture medium generated from amniotic membrane of human placenta (hAMSC) blocked B-cell differentiation, with an increase of the proportion of mature B cells, and a reduction of antibody-secreting cell formation [92]. Additionally, MSC-released NO, inhibited B cells, decreased antibody production by $CD5^+$ B cells and prevented leukocyte recruitment by adhesion, extravasation, and chemotaxis mechanisms [85,93].

All in all, and although the mechanisms behind this type of interaction are not fully elucidated, there is great evidence of the beneficial action of MSC-CM on the different cells of innate and adptive immunity.

Angiogenic properties of MSC secretome

Angiogenesis is a process controlled by a balance between stimulatory and inhibitory signals with a role in the angiogenic process. VEGF, FGF-2, PDGF, SDF1, CXCL-1, RANTES, MCP-1 and M-CSF are factors present in the MSC secretome known for inducing angiogenesis by binding to their corresponding receptors on endothelial cells and activating the signaling pathways p38/MAPK, PI3K/AKT, MEK/ERK [94–96]. The angiogenic potential of MSC secretome has been shown in the scope of different diseases, such as neurological diseases of traumatic brain injury and Alzheimer's disease [97,98], cardiac diseases and bone tissue regeneration, among others. In fact, osteogenesis is closely related with vascularization due to cell-to-cell communication between vascular endothelial cells and osteoblasts [99]. For example, the growth factor VEGF acts on vascular endothelial cells, enhancing not only angiogenesis, but also bone development [99]. The chemoattractant potential of MSC secretome for endothelial cells was also demonstrated, as well as the capacity to these cells to induce the formation of capillary-like structures in the presence of MSC secretome [96]. In cardiac diseases, MSC-EVs also enhanced blood flow recovery and reduced infarct size in animal models [97,100].

Anti-tumoral properties of MSC secretome

MSCs have also been regarded as possible cancer therapeutic agents since they exhibit an intrinsic ability to migrate towards tumors. In the pro-inflammatory tumor microenvironment (TME) occurs the expression of a plethora of chemokines and cytokines, namely VEGF, FGF, PDGF, CCL5 and IL-8, that induce the migration of MSCs towards the tumor in an orchestrated manner by creating a chemokine concentration gradient [101]. Yet, the interactions between MSCs and tumor cells remains poorly understood with controversial results reporting a pro-tumorigenic role. The

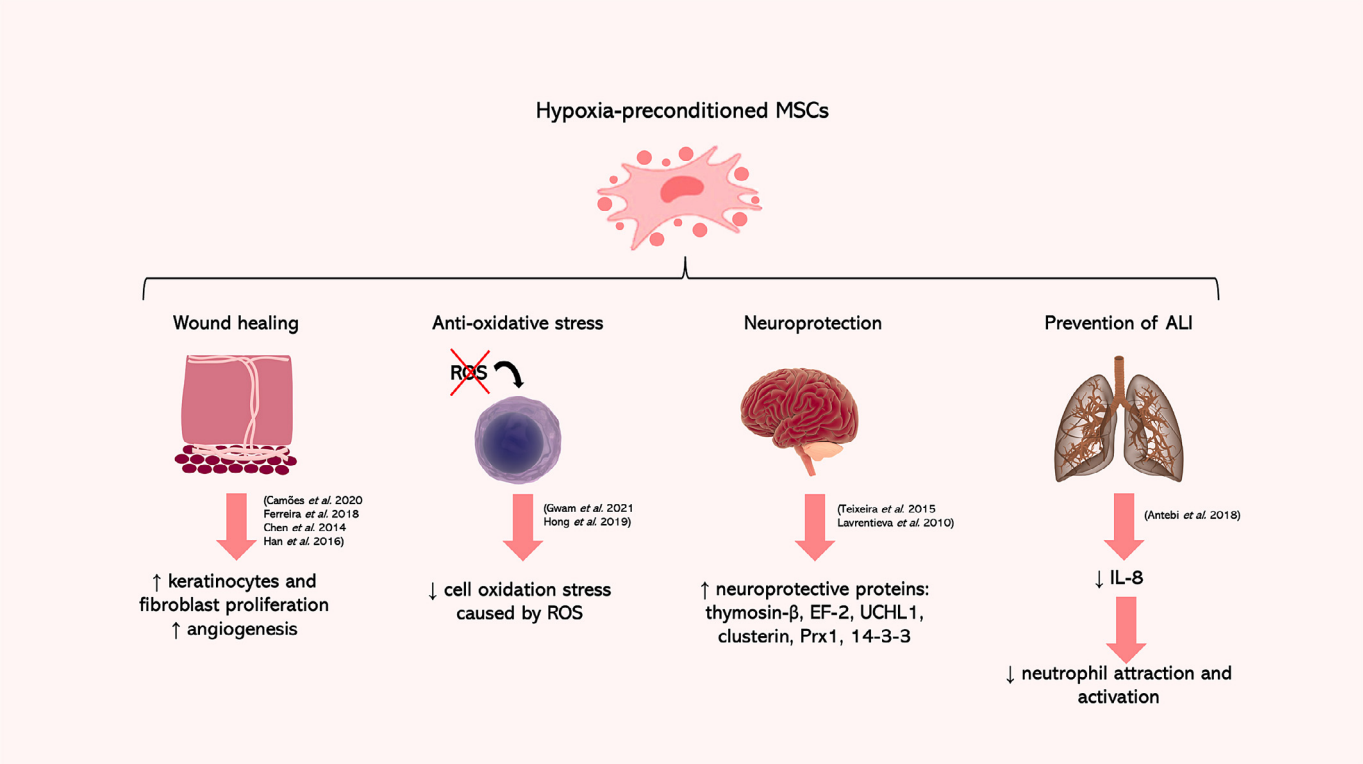


Fig. 2. Benefits of hypoxia-preconditioned MSCs compared to normoxia MSCs. Hypoxia-preconditioned MSC-derived secretome has shown to induce keratinocytes and fibroblast proliferation as well as angiogenesis by releasing high levels of angiogenic factors, therefore enhancing the process of wound healing. MSC-derived secretome obtained in hypoxia conditions can reduce the concentration of ROS due to low levels of O_2, thus reducing the cellular oxidative stress. Regarding neuroprotection, MSCs obtained under these conditions have shown to release high levels of proteins with a neuroprotective role and, in the context of acute lung injury, have revealed to reduce neutrophil activation by reducing the levels of IL-8.
Abbreviations: ALI: acute lung injury; EF-2: eukaryotic elongation factor 2; IL-8: interleukin 8; MSCs: mesenchymal stem cells; Prx1: peroxiredoxin 1; ROS: reactive oxygen species; UCHL1: ubiquitin C-terminal hydrolase L1.

methodology concerning MSC application in cancer studies, along with the source of MSCs and/or the type of cancer cells, may have impact on the outcome. The use of MSCs themselves may also have a contribution given the reports of their predisposition to differentiate into cancer-associated fibroblasts, which are correlated with tumor growth, invasion and metastasis [102,103]. Conversely, an overall anti-tumoral effect of MSCs is observed when using their derived secretome on tumor cells, highlighting the need to understand the mechanisms behind this differential effect. Indeed, many authors have been reported the effect of the secretome of MSCs in tumor cell proliferation and invasion *in vitro* and *in vivo* [104–107]. MSC-derived secretome promote cell cycle arrest and induction of apoptosis through the upregulation of pro-apoptotic *Bax* gene and negative regulator of cell cycle such as p21, and downregulation of anti-apoptotic *Bcl2*, Survinin and *Xiap* genes [108–112]. Moreover, the disruption of the cancer-associated signaling pathways, such as PI3K/AKT [107,113], via upregulation of PTEN, and of WNT/β-catenin signaling pathway by MSCs-secreted antagonist of the Wnt signaling DKK-1 [114] contributes to the inhibition of tumor cell migration, invasion, and survival [114–118]. Other factors have been also associated with cancer suppression mediated by MSCs, namely TNFSF14, FLT-3, IP-10 and LAP [119]. Still, MSC secretome has been also shown to decrease doxorubicin-induced cytotoxicity in non-tumor cells, without compromising doxorubicin chemotherapeutic profile in malignant cells, which suggests its potential use as a chemotherapy adjuvant to reduce off-target side effects [43].

Current trends to ameliorate MSC secretome

Multiple studies exploring strategies of modulating environmental factors with the potential to enhance the beneficial effects of secretome-contained biomolecules have been performed, namely via preconditioning [18,54,120]. It involves the *in vitro* modulation of the secretome produced by MSC by exposing these cells to specific factors that usually mimic the tissue damage microenvironment [73], namely to hypoxia, inflammatory or disease-specific cytokines and/or pharmacological compounds, or even by using 3-dimensional (3D) cell culture systems [54,120,121].

Hypoxia-preconditioned MSCs

Although MSCs are usually cultured under normoxia conditions, with an oxygen pressure of 21 %, the oxygen tension within tissues is typically hypoxic, ranging between 1–7 % and 10–15 % in the BM and AT, respectively [37]. Several studies have already demonstrated that hypoxia-preconditioned MSCs can frequently potentiate the therapeutic potential [122] of different types of MSCs, including BM-, UC- and AT-MSCs.

In the context of cutaneous wound healing, it has been demonstrated that the secretome obtained from hypoxia preconditioned-BM-MSCs upregulated the levels of FGF-2, IGF-1, TGF-β, IL-1β, IL-6 [123] and IL-8 [122] and enhanced multiple key processes to promote wound healing, including keratinocyte,

fibroblast and endothelial cells proliferation and migration, as well as angiogenesis (Fig. 2) [38,120,123]. On the other hand, hypoxia-preconditioned-UC-MSCs have enhanced angiogenesis-related genes, including *Cox-2*, *Vegf* and *Tie-2*, reduced the proinflammatory genes *Il-1* and *Il-20* and increased the anti-inflammatory gene *Tgf-β* compared to normoxic conditions, in both *in vitro* and *in vivo* mouse models of ischemic hindlimb [124].

Moreover, hypoxia preconditioning in BM-MSCs was demonstrated to facilitate liver regeneration after 85 % hepatectomy in rat models, with a significant increased cyclin D1, VEGF, PCNA-positive hepatocytes, liver weight/body weight ratio, serum albumin levels and cell survival [125]. In addition, in thioacetamide (TAA)-treated AML12 mouse hepatocytes, Hong *et al.* reported that hypoxia-preconditioned secretome derived from AT-MSCs promoted the downregulation of intracellular levels of oxygen reactive species (ROS), often dysregulated in hepatic disorders, and upregulated SOD, glutathione peroxidase, and catalase [126,127].

In neurological diseases context, proteomic analysis revealed that hypoxia enhances the expression of proteins [128–130] associated to neuroprotection, neural survival, differentiation, and axonal and neurite growth, when compared to the secretome obtained from normoxia-cultured MSCs [128]. These proteins include thymosin-β, EF-2, UCHL1, clusterin, Prx1 and 14-3-3 (Fig. 2) [128]. Yet, higher concentrations of oxygen were associated with higher quantities of ROS and, thus, to a higher cell toxicity [128–130].

Furthermore, the pre-treatment of BM-MSCs with a hypoxia inductor such as deferoxamine showed to enhance osteogenic differentiation of these cells and extracellular matrix mineralization through the decrease of histone methylation and increase of histone acetylation [131], showing that a priming strategy that indirectly leads to hypoxia can act by epigenetic modifications.

Inflammatory-preconditioned MSCs

Inflammatory stimuli are often present in pathological microenvironments [132]. Thereby, specific inflammatory cytokines stimuli might be a preconditioning strategy where MSCs tend to produce immunoregulatory factors as a survival mechanism to help the microenvironment to fight against the excessive inflammation, thus improving the therapeutic properties of MSC-derived secretome [133].

The immunoregulatory role of MSCs was shown to be stimulated in the presence of one or more cytokines, such as IFN-γ, IL-1α, IL-1β or TNF-α, all released by macrophages [133–135]. This type of priming commonly induce MSCs into a negative feedback mechanism that results on release of anti-inflammatory mediators, such as TSG-6 [73], or to express larger levels of immunomodulatory mediators, including IDO and nitric oxide synthase (iNOS) [135], as well as some specific ligands for chemokine receptors having a role in the chemotaxis of T lymphocytes, such as CXCR3 and CCR5. Consequently, the recruitment of T cells to the vicinity of MSCs and secreted immunosuppressive molecules was shown to downregulate the T cells inflammatory state [133,135].

Indeed, IFN-γ-primed MSCs have demonstrated to block T cell proliferation in a dose-dependent manner and to abrogate the production of IFN-γ, IL-2 and TNF-α, three cytokines with a role in T cell-derived immune response [136]. IFN-γ and TNF-α has revealed to potentiate both the antioxidant and anti-inflammatory activity of AT-MSCs, by reverting the hippocampal neuroinflammation and oxidative stress induced by alcohol [3]. In addition, LPS or TNF-α preconditioning activated toll-like receptor 4 (TLR-4) and TNF receptor 1 (TNFR-1), leading to the upregulation of NF-κB signaling pathway and, subsequently, to an increase expression of COX-2 which, in turn, triggers PGE_2 secretion [73]. TNF-α, in particular, was associated to the production of VEGF and to potentiate angiogenesis in a time-dependent manner, or to enhance the re-epithelization and wound resolution [96,137–140]. Overall, IFN-γ or TNF-α preconditioning MSC-secretome showed enhanced immunosuppressive [141,142] and pro-angiogenic effects [96,137–140,143].

On the other hand, preconditioning of AT-MSCs with LPS promoted liver regeneration in partially hepatectomized mice by enhancing HGF, IL-6, TNF-α and VEGF expression levels, while reducing the serum levels of ALT, AST, IL-6 and TNF-α [144]. This result indicates that LPS-preconditioning-MSC-derived secretome has better liver regeneration and anti-inflammatory properties compared to non-primed MSC-secretome [144].

Priming MSCs with IL-1α and IL-1β led to a pro-neurotrophic and anti-inflammatory secretome [145], with increased levels of G-CSF, a neurotrophic factor, given its ability to enhance synaptogenesis, angiogenesis, neuroprotection and neurogenesis [145]. BM-MSCs treated *in vitro* with IL-1β have also demonstrated a high capacity to recruit both monocytes and granulocytes but also to increase the levels of several factors, such as TNF-α, IL-8 and CSF2, with a role in monocytic differentiation [146].

3D culture preconditioned MSCs

Another strategy for preconditioning MSCs appears to be by culturing MSCs in 3D culture systems, a system that further mimics the complexity of physiological conditions, namely by allowing better cell-to-cell and cell-to-extracellular matrix (ECM) interactions (Fig. 3) [8,38,39]. Overall, spheroid cultures of MSCs were shown to promote greater immunomodulatory, pro-angiogenic, anti-fibrotic and anti-apoptotic properties, when compared to traditional 2D cultures [120], also associated with higher levels of CXCL12, FGF-2, G-CSF, HGF, IGF-1, IL-6, TGF-β1, and VEGF, along with increased secretion of several anti-inflammatory mediators, among others (Fig. 3) [18,147–150].

In the context of inflammatory arthritis, our group has demonstrated that the secretome obtained from 3D cultures, i.e. three-dimensional conditioned media (CM3D), was able to counteract the manifestations typifying rheumatoid arthritis, possible due to the enrichment of some trophic factors with anti-inflammatory properties, such as FGF-2, IL-10 and PDGF-BB [8]. Likewise, Bhang and colleagues showed that CM3D, this time derived from AT-MSCs, contained ∼30-fold higher concentrations of the pro-angiogenic factors CXCL12, FGF-2, HGF and VEGF, resulted in higher angiogenic effects and a marked increase of endothelial cell growth in an in vivo animal model of hindlimb ischemia [147]. Furthermore, CM3D has enhanced the blood perfusion on the ischemic limb and the recruitment/homing of bone marrow mononuclear cells (BMMNCs) from the BM to the ischemic region [147].

In the scope of ischemic stroke, AT-MSC-derived CM3D-treated animals revealed a better performance of the Rotarod test, a motor function assay, as well as a significant reduction of the infarction volume. In fact, the same study revealed higher levels of the pro-angiogenic factor TGF-β1 and, on the other hand, a decrease in the apoptosis of all neural cell types, most markedly in microglia, suggesting a decreased microgliosis in the treated animals compared with the non-treated animals [150].

Regarding wound healing, we have also previously shown that the secretome derived from UC-MSC spheroids has higher levels of FGF-2, G-CSF, HGF, IL-6 and TGF-β1, when compared to two-dimensional conditioned media (CM2D). These results indicated that fibroblast-mediated ECM, angiogenesis and vasculogenesis, processes implicated in the wound healing process, are indeed improved by 3D culture-preconditioning strategies [8]. Accordingly, the presence of the antifibrotic factors, including IGF-1, IL-6 and HGF in CM3D protected hepatocytes *in vitro* from cell injury and apoptosis and ameliorated hepatic fibrosis and *in vivo* liver function (Fig. 3) [149].

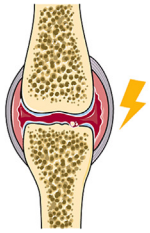
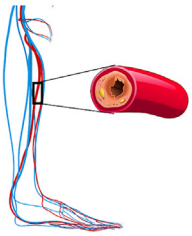
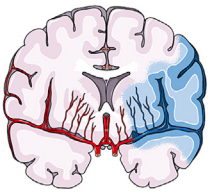
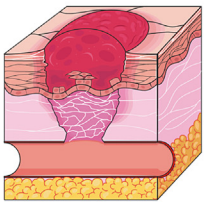
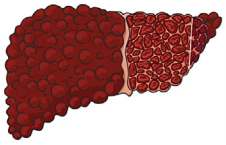
	Rheumatoid arthritis	Ischemic hindlimb	Ischemic stroke	Wound healing	Hepatic fibrosis
Pathology					
Higher content factors of MSC CM3D *vs.* MSC CM2D	FGF-2, IL-10, PDGF-BB (Miranda *et al.* 2019)	CXCL12, FGF-2, HGF, VEGF (Bhang *et al.* 2014)	TGF-β1 (Cho *et al.* 2012)	FGF-2, G-CSF, HGF, IL-6, (Miranda *et al.* 2019)	IGF-1, IL-6 and HGF (Zhang*et al.* 2016)
Other obtained results with MSC CM3D *vs.* MSC CM2D	Higher motogenic activity over chondrocytes	Increase in SM positive α-actin-stained microvessels, great increase in endothelial cell growth, homing of BMMNCs to the ischemic region	Improved motor function (Rotarod test), reduction in ischemic area, increase in $CD31^+$ microvessels in the penumbra area	Improved in fibroblast-mediated ECM, angiogenesis and vasculogenesis	Protection of hepatocytes from cell injury and apoptosis and ameliorated hepatic fibrosis and liver function

Fig. 3. The main factors involved in the greater effects of MSC-CM3D in different pathologies *(This figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).*
Abbreviations: BMMNCs: bone marrow mononuclear cells; CXCL12: C-X-C motif chemokine ligand 12; ECM: extracellular matrix; FGF-2: fibroblast growth factor 2; G-CSF: granulocyte colony-stimulating factor; HGF: hepatocyte growth factor; IGF-1: insulin-like growth factor; IL-6: interleukin 6; IL-10: interleukin 10; MSCs: mesenchymal stem cells; MSC-CM2D: MSC-derived two-dimensional conditioned media; MSC-CM3D: MSC-derived three-dimensional conditioned media; PDGF-BB: platelet-derived growth factor BB; SM: smooth muscle; TGF-β1: transforming growth factor 1; VEGF: vascular endothelial growth factor.

Genetic-engineered MSCs

Since MSCs can be easily transfected, the genetic manipulation of MSCs *in vivo* can be an additional approach to improve the therapeutic potential of their secretome. As an example, MSCs overexpressing the survival protein AKT release higher levels of the paracrine factors FGF-2, HGF, IGF-1 and VEGF [151]. Indeed, VEGF promotes angiogenesis and restoring tissues, further highlighting its pivotal role in several tissue injuries, namely in myocardial infarction, wound healing, ischemic stroke, among others [151].

In the neurological field, neurotrophin-3-transfected BM-MSCs-CM has shown to reduce the apoptosis of motor neurons in an animal model of spinal cord injury (SCI) [152,153].

The engineered BM-MSC to overexpress HGF have also demonstrated promising results in liver [154] and kidney [155] regeneration in rat models of disease. Regarding liver diseases, MSC overexpression with c-met and CXCR4 improved homing to the livers of acute liver failure rodent models [156,157], resulting in enhanced liver function and increased survival rates.

MSC secretome in regenerative medicine

The application of MSC-derived secretome has been studied towards different pathologies by counteracting inflammation while inducing tissue regeneration namely in lung, hepatic and brain tissues. Nevertheless, this cell-free approach has also been reported with beneficial effects for other pathologies, including bone tissue, cardiovascular, intestinal and renal diseases [158–170] (Table 2).

MSC secretome in respiratory diseases

There are several studies revealing the beneficial effects of MSCs in lung fibrosis and acute lung injury [74,171–178] (Fig. 4). Lung fibrosis is characterized by the replacement of airy alveoli by stromal cells, including myofibroblasts. Myofibroblasts produce ECM proteins, including misfolded collagen, leading to lung fibrosis and respiratory sequelae [176]. Pulmonary fibrosis can derive from multiple lung diseases, including lung infection, autoimmune diseases, chronic inflammation, and idiopathic causes [176,177]. Moreover, acute respiratory distress syndrome (ARDS), and its milder form acute lung injury, encompass a group of lung diseases with an exacerbated pro-inflammatory response that impairs the integrity of the alveolocapillary barrier with a consequent increased lung protein permeability and pulmonary edema and fibrinogenesis. The impaired alveolar fluid clearance hampers gas exchange and triggers hypoxemia and hypercapnia [172,173]. Patients with ARDS frequently have multi-organ failure and the most often etiology is sepsis syndrome. Although ARDS can have different origins, sepsis from both pulmonary and non-pulmonary origins is the most frequent cause [173]. Due to the lack of an effective treatment against ARDS, the use of MSCs offers a promising therapeutic approach against ARDS and acute lung injury [172]. Importantly, the burden of pulmonary fibrosis due to SARS-CoV-2 infection is also expected to increase [177].

Particularly, the increase of IL-1β concentrations in lung airways has been associated with neutrophil accumulation in lungs [74]. Hence, using IL-1RA can be a good strategy of attenuating acute lung injury. In fact, IL-1RA-producing MSCs has completely reverted lung inflammation in mice by suppressing production of

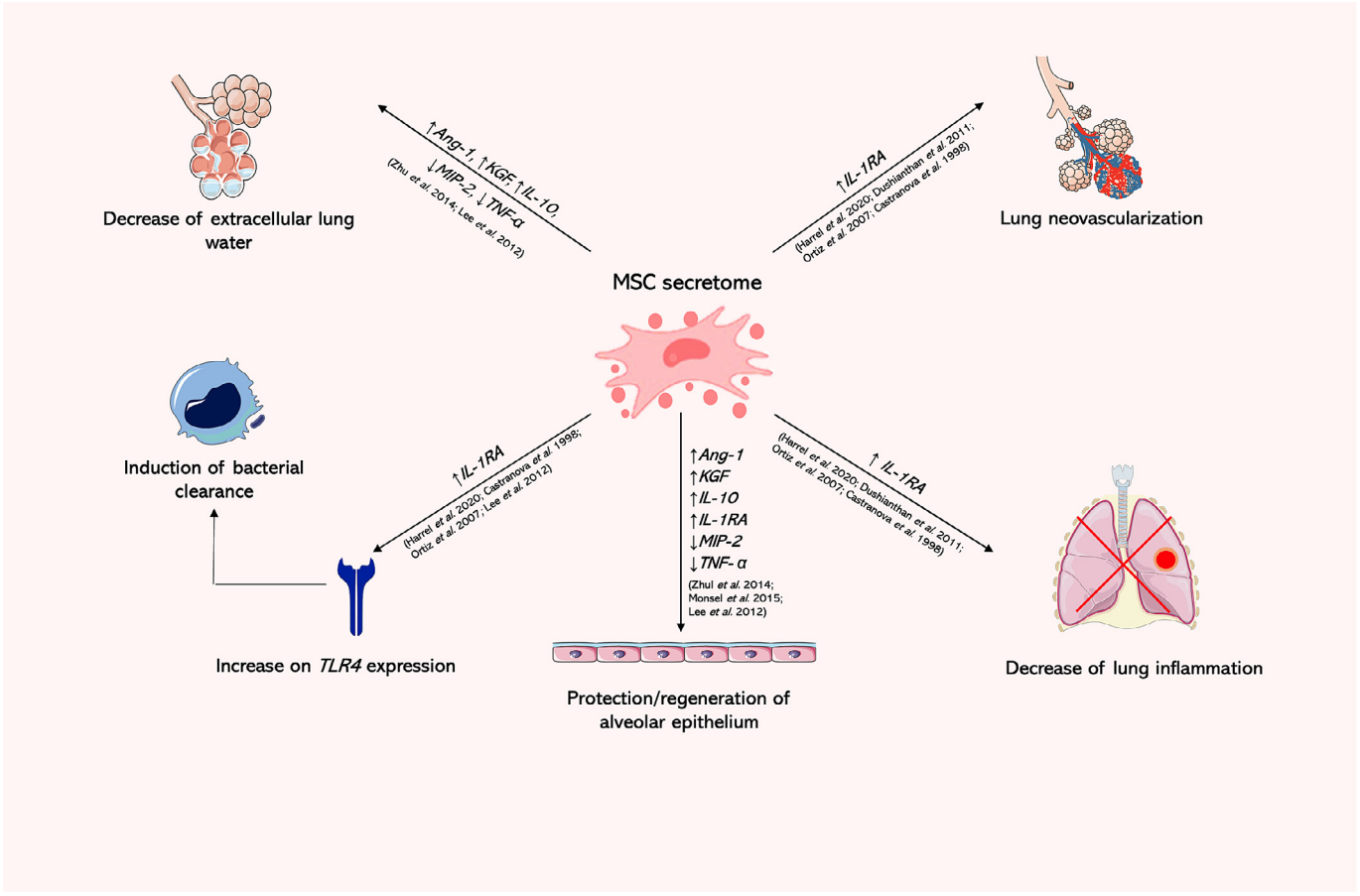


Fig. 4. The role of main players of MSC-derived secretome on the several features that can be present on respiratory diseases. *(This figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license).*
Abbreviations; Ang-1: angiopoietin 1; KGF: keratinocyte growth factor; IL-10: interleukin 10; IL-1RA: interleukin 1 receptor antagonist; MIP-2: macrophage inflamamtory protein 2; MSCs: mesenchymal stem cells; TNF-α: tumor necrosis factor alpha; TLR4; toll-like receptor 4.

IL-1α, IL-1β and TNF-α by alveolar macrophages [74,175]. Furthermore, IL-1RA was shown to upregulate VEGF and, thus, indirectly enhance neovascularization and the regeneration of injured alveolar type II epithelial cells [174], pivotal for alveolar epithelium regeneration after lung injuries [179]. IL-1RA was also shown to induce TLR-4 expression, crucial for bacterial clearance [74].

Some authors have suggested that the beneficial effects of MSCs administration derive from the reduction of TNF-α and MIP-2 in both the bronchoalveolar lavage fluid (BALF) and plasma and, simultaneously, from the increase of IL-10 [171]. It has been also found that MSCs segregates KGF and angiopoietin-1 (Ang-1), two soluble factors that revealed to protect alveolar epithelium and endothelium besides promoting fluid clearance [172].

Importantly, as detailed in Table 3, MSC-derived-EVs have also been implicated in the treatment of these pathologies. A reduction in parameters of lung inflammation, as assessed by the decrease of *i)* bacterial growth [180], *ii)* lung proteins to BALF [68,180], *iii)* excessive response of neutrophils [68] and *iv*) pro-inflammatory mediators, such as HIMF/FIZZ-1, IL-6, MCP-1, MIP-2 and TNF-α [181].

Recent studies also found that AT-MSC-EVs attenuated lung fibrosis in an animal model (bleomycin-induced damage), demonstrating a decrease in fibrotic damage, while the soluble part of the secretome did not have the same effect [176].

MSC secretome in liver diseases

Liver diseases are among the leading causes of death worldwide. A punctual liver injury, either of metabolic (e.g., type II diabetes, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatatis (NASH)), viral (e.g., hepatitis B), chemical or tumoral origin activates the liver's unique intrinsic regenerative hepatic response, intending to re establish homeostasis [182]. However, repeated injuries may lead to fibrosis, with inflammatory activation and progressive levels of hepatic parenchymal dysfunctions. Increasing degrees of insufficiency result in liver failure and the only effective treatment for end-stage disease is liver transplantation. Currently, liver transplantation is the second most common solid organ transplantation, yet the current rate of transplantation only meets 10 % of the global need [183]. Therefore, many groups have focused their research in using MSCs as a therapeutic approach, particularly to induce liver's regeneration after injury [184].

Inflammation is one of the first signs appearing in the injured liver, such as NAFLD, contributing to fibrosis and failure of the hepatic function. Thus, MSC immunomodulatory activity may play a role in liver pathologies. In particular, BM-MSC-CM induced a decrease in IL-1β, IL-6, MCP-1, MIP-2 and TNF-α and resulted in the reduction of neutrophil infiltration and Kupffer cell activation

Table 3
Preclinical studies demonstrating the beneficial effects of MSC-derived EVs on respiratory diseases.
Abbreviations: BALF: bronchoalveolar lavage fluid; BM-MSCs: bone marrow-mesenchymal stem cells; CFUs: colony-forming units; FAPa: fibroblast activation protein alpha; hESC-IRMCs: human embryonic stem-cells derived MSCs-like immune and matrix regulatory cells; HIMF/FIZZ1: hypoxia-induced mitogenic factor/found in inflammatory zone 1; Ho-1: heme oxygenase 1; IFN-γ: interferon-gamma; IL-6: interleukin-6; KGF: keratinocyte growth factor; MCP-1: monocyte chemoattractant protein-1; MIP-2: macrophage inflammatory protein 2; Nox4:NADPH oxidase 4; Nrf2: nuclear factor erythroid 2–related factor 2; PBS: phosphate buffered saline; TLR3: toll-like receptor; TNF-α: tumor necrosis factor alpha; UC-MSCs: umbilical cord-mesenchymal stem cells.

Source of EVs	Disease	Animal model	Sample	EVs effects	Treatment outcomes	Ref
UC-MSCs	Hypoxia-induced pulmonary hypertension	*In vivo* mouse model of hypoxia-induced pulmonary hypertension	BALF	↓ MCP-1, HIMF/FIZZ1, IL-6	Inhibition of parenchyma injury, vascular remodeling and right ventricle hypertrophy	[181]
hBM-MSCs	Acute lung injury	*E. coli* endotoxin-induced acute lung injury model	BALF	↓ MIP-2	Reduction of extravascular lung water, protein levels in BALF and pulmonary oedema Reduction of neutrophils influx	[68]
hBM-MSCs primed with a TLR3 agonist vs. hBM-MSCs non-primed or *vs.* PBS	Acute lung injury	*E. coli* endotoxin-induced acute lung injury model	BALF	↓ number of bacterial CFUs (*vs.* hBM-MSCs non-primed) ↑ KGF (*vs.* hBM-MSCs non-primed)	Reduction of lung inflammation, protein permeability	[180]
			Blood	↑ bacteria phagocytosis by monocytes (*vs.* PBS)	Reduction of bacterial growth	
IFN-γ-primed hUC-MSCs vs. non-primed hUC-MSCs	Acute lung injury	E. coli endotoxin-induced acute lung injury model	n.a.	↓ alveolar protein concentration	Attenuated increase in alveolar permeability	[265]
			BALF	↓ [TNF-α]	Reduction of pro-inflammatory cytokine response	
AT-MSCs	Pulmonary fibrosis	Bleomycin-induced lung damage animal model	n.a.	Average dynamic of −40 %	↓ lung fibrotic damage	[176]
			n.a.	↓ collagen deposition		
			Lung tissue	↓ of α-SMA$^+$ myofibroblasts and FAPa$^+$ myofibroblasts precursors		
hESC-MSC-IRMCs	Pulmonary fibrosis	Bleomycin-induced lung damage animal model	Lung tissue	↓ Nox4 and ↑ Nrf2 and Ho-1 expression	↓ lung fibrosis and oxidative stress and inflammation	[178]
			Lung tissue	↓ α-SMA$^+$ cells		
			Lung homogenates	↓ [IL-1β], [IL-6] and [TNF-α]	Reduction of pro-inflammatory cytokine response	

(Fig. 5) [185]. Other MSC-secreted cytokines, such as TGF-β, HGF, IL-10 and IDO, were also shown to be capable of modulating the immune system, by acting on T cell and antigen presenting cell (APC) proliferation which consequently decreases the exacerbated inflammatory response in liver diseases [186–194]. In addition, the anti inflammatory molecule annexin-A1 (ANXA1) present in amniotic fluid-derived MSC-secretome ameliorated liver damage in a CCl_4 induced liver injury mouse model by inducing hepatic progenitor proliferation, migration and differentiation while reducing inflammation [195]. Likewise, UC-MSC-CM ameliorated liver dysfunction and reduced lipid accumulation, pro-inflammatory cytokine levels and the presence of apoptotic markers in a mice model of NAFLD [196,197].

Another consequence of the inflammatory activation during hepatic injury is the hepatic fibrosis. In normal hepatic tissue, hepatic stellate cells (HSCs) are usually at a quiescent state at the space of Disse and store vitamin A. However, upon injury signals, besides hepatocyte damage, there is an infiltration of immune cells that promotes *trans*-differentiation of HSCs into myofibroblasts, responsible for collagen type I production and deposition [198]. Fibrosis is also commonly associated with hepatic viral infection, alcoholism, hepatotoxic drugs, and even metabolic and autoimmune diseases. Therefore, an important step towards liver regeneration is the repair of fibrosis caused by the activation of HSCs. In fact, IL-10, IL-1RA, HGF and TNF-α secreted by MSCs were demonstrated to inhibit collagen synthesis and to induce HSC apoptosis (Fig. 5) [74,191,199,200]. Specifically, inhibition of TGF-β1/Smad pathway is associated to HSC apoptosis [201]. In particular, TGF-β3 and HGF secreted by AT-MSCs induced G_0/G_1 arrest of HSC growth through the upregulation of $p27^{Kip1}$ and $p21^{Cip1}$ expression and downregulation of cyclin D1 [192]. Regarding IL-1RA, this receptor antagonist was also shown to downregulate IL-1β and TNF-α in liver macrophages [74,200]. Furthermore, MSCs can revert HSC activation by inhibiting a1(I) procollagen and a1(III) procollagen expression, or by downregulation of the phosphorylation of MAPK and ERK1/2 [192]. Moreover, MSCs can modulate fibrosis by enhancing the expression of matrix metalloproteinases (MMPs) and downregulating the expression of tissue inhibitors of MMPs, resulting in the regression of liver fibrosis [202,203].

MSC trophic factors also have positive effects in hepatocyte proliferation and migration, while reducing hepatocellular apoptosis by a wide range of factors [204]. For example, IL-6 secreted from BM-MSCs was found to induce FGL1 expression in hepatocytes from CCl_4-treated mice resulting in an anti-apoptotic effect [205]. Soluble factors such as SDF-1, IGF-1, Nrf-2, HIF, HO-1 and VEGF were also reported to downregulate pro-apoptotic factors, such as Bax, and increase the levels of the anti-apoptotic protein Bcl-2 and the activity of antioxidant proteins [61,206]. Surprisingly, in a model of liver injury induced by ischemia/reperfusion, it was also demonstrated that UC-MSCs-CM suppressed hepatocyte apoptosis through the ability of improving the mitochondrial quality control [207], namely by upregulating PINK1-dependent mitophagy [208].

Furthermore, MSCs can induce new vasculature from pre-existing blood vessels [39]. The angiogenic potential of MSCs was exerted through the secretion of angiopoietins (ANGPTs), HGF, IGF-1, VEGF-A, VEGF-R1 and VEGF-R2, contributing to endothelial cell proliferation and improvement of tissue vascularization [209,210]. Therefore, it has been shown that the combination of MSCs with EGF, FGF-2, IGF-1 and VEGF induced G-CSF, HGF, IGFBP-2 and IL-8 expression in MSCs, enhancing their therapeutic

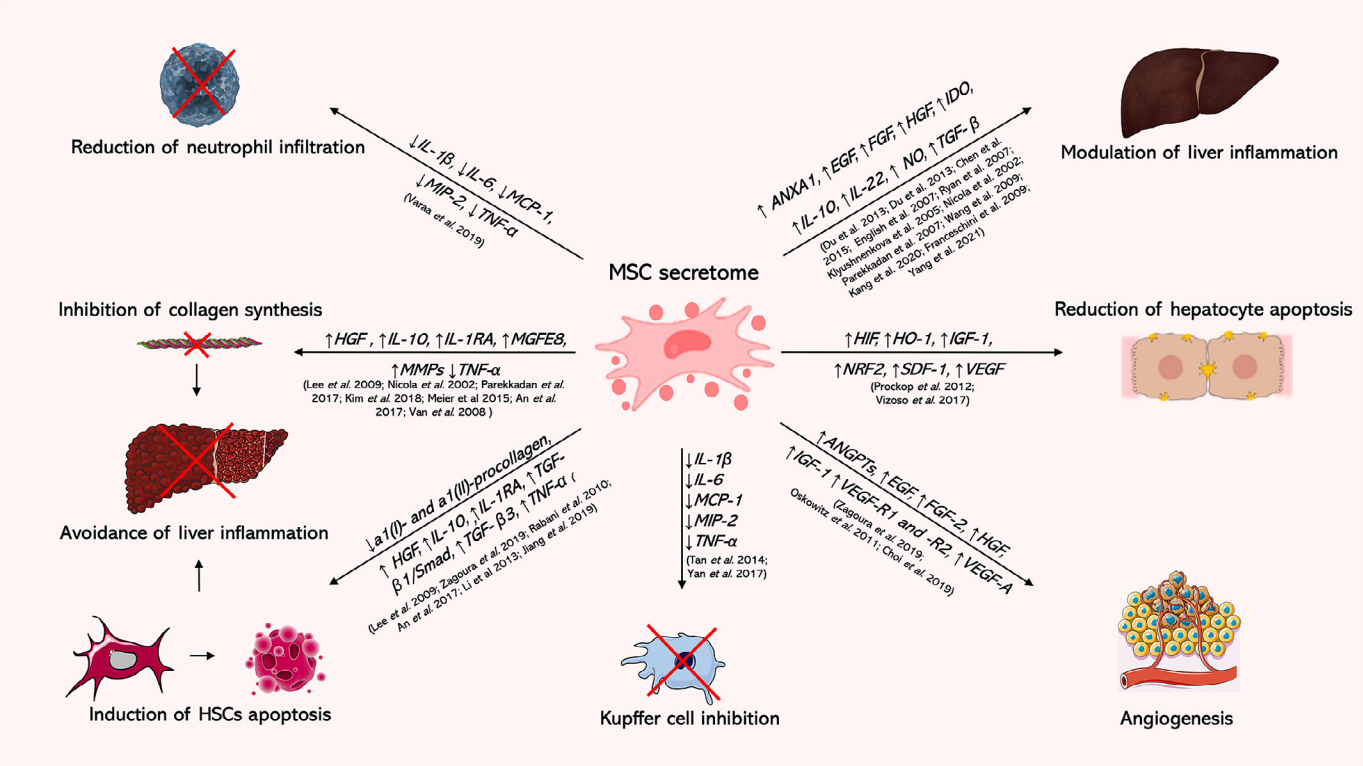


Fig. 5. The main mechanisms and factors involved in the liver regenerative potential of MSC-derived secretome. (*The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license*).
Abbreviations: ANXA1: annexin A1; ANGPTs: angiopoietins; EGF: epidermal growth factor; FGF-2: fibroblast growth factor 2; HGF: hepatocyte growth factor; IDO: indoleamine-pyrrole 2,3-dioxygnease; IGF-1: insulin-like groth factor; IL-1β: interleukin 1 beta; IL-6: interleukin 6; IL-10: interleukin 10; IL-22: interleukin 22; IL-1RA: interleukin 1 receptor antagonist; MCP-1: monocyte chemoattractant protein 1; MIP-2: macrophage inflammatory protein 2; MGFE8: milk fat globule epidermal growth factor 8; MMPs: matrix metalloproteinases; MSC: mesenchymal stem cells; Smad: mothers against decapentaplegic; TGF-β: transforming growth factor beta; TNF-α: tumor necrosis factor alpha; VEGF-A: vascular endothelial growth factor A; VEGF-R1/R2: vascular endothelial growth factor receptor 1/2.

function in a TAA induced liver fibrosis model by improving liver function and regeneration [197,211].

Interestingly, MSC-derived EVs have also demonstrated to ameliorate liver fibrosis, decrease of hepatic inflammation, collagen deposition, hepatocyte apoptosis, reduce the epithelial-to-mesenchymal transition of hepatocytes and consequently promote liver function recovery [212,213] (Table 4).

Overall, MSC-derived secretome has demonstrated to have immunomodulatory, anti-apoptotic, anti-fibrotic and angiogenic effects, all promoters of hepatocyte function and proliferation, resulting in the amelioration of liver injury.

MSC secretome in neurological diseases

Although until recently it was thought that the central nervous system (CNS) was fixed and incapable of regeneration, nowadays it is well-established that neural plasticity persists throughout life in humans [214,215]. This process occurs by different mechanisms, including the regulation of synaptogenesis in mature neurons but also the function of supportive glial cells, including microglia. Interestingly, neuroplasticity is also assured by the presence of neural stem cells (NSCs) and neural progenitor stem cells (NPCs) in specific niches of the adult nervous system [216], which in turn constitute a reservoir for cell lost during physiologic cell turnover and after brain damage [217]. Of note, NSCs of the adult mammalian are organized mainly in two brain neurogenic niches: the subventricular zone (SVZ) of the walls of the lateral ventricles [215] and the subgranular zone (SGZ) of the hippocampal dentate gyrus [218]. In these neurogenic niches, NSCs can differentiate into neuronal cells, astrocytes or oligodendrocytes [219]. However, the half-life of these cells as well as all mechanism associated to neuroplasticity drops sharply with ageing, being implicated in several aging-related cognitive deficits and impairments, such as Alzheimer's disease and major depressive disorder [220].

Since MSC-derived secretome has immune and neuroregulatory properties, exposure of neural cells, including NSCs to MSC-derived secretome could also represent a novel and feasible strategy to enhance the proliferation and differentiation of these cells, delaying a wide range of neurological and psychiatric conditions associated with NSC depletion (Fig. 6) [54,221,222]. Indeed, MSCs were already shown to be capable of expressing a wide range of neuroregulatory molecules that regulate NSCs fate, axon guidance, neural cell adhesion, neurite growth factors, neurotransmitter receptors, and neurotrophic factors [223]. Besides neuroregulatory properties, MSC-derived secretome was also shown to decrease the excessive neuroinflammation observed in most brain injuries. Moreover, MSC-derived factors, such as and BDNF, FGF-2 GDNF, IGF, NGF and neurotrophin-3, revealed to be important growth factors for neuronal/glial cell population [120,214,222,224–226] (Fig. 6). The administration of MSCs-derived EVs has also been explored in the scope of different neurological and psychiatric diseases, including ischemic stroke, Parkinsońs disease, nerve pain and even depression (Table 5).

Although preliminary, AT-MSCs-derived CM was used in a mouse model of amyotrophic lateral sclerosis ALS [227], showing that systemic administration of AT-MSCs-CM can attenuate the progression of late-stage ALS mice and prevent neuromuscular junctions' denervation in early stages of ALS [227].

Table 4
Studies demonstrating the beneficial effects of MSC-derived EVs on liver diseases.
Abbreviations: ALP: alkaline phosphatase; ALT: alanine aminotransferase; ALF: acute liver failure; AT-MSCs: adipose tissue-derived mesenchymal stem cells; aSma: alpha-smooth muscle actin; AST: aspartate aminotransferase; Bcl2: B-cell lymphoma 2; Bcl-XL: B-cell lymphoma-extra large; Bax: Bcl-2 associated X-protein; Col1a: collagen type I alpha; D-GalN: D-galactosamine; GGT: gamma-glutamyl transferase; hBM-MSCs: human bone marrow-mesenchymal stem cells; hfMSCs: human fetal mesenchymal stem cells; HSCs: hepatic stellate cells; hUC-MSCs: human umbilical cord-mesenchymal stem cells; hESC: human embryonic stem cells; IL: interleukin; iNOS: inducible nitric oxide synthase; LPS: lipopolysaccharide; MDA: malondialdehyde; MIP-2: macrophage inflammatory protein 2; miR-17: miRNA 17; NF-κB: nuclear factor kappa B; PCNA: proliferating cell nuclear antigen; STAT3: signal transducer and activator of transcription 3; TAA: thioacetamide; TAMH cell line: immortalized mouse hepatocyte cell line from transgenic MT42 male mice over-expressing TGF-α. It is a metabolically competent liver cell line that reproduces features of cytotoxicity; Timp1: TIMP metallopeptidase inhibitor 1; TGF-β1: tumor growth factor beta 1; Tnf-α: tumor necrosis factor alpha.

Source of EVs	Disease	Animal model	Sample	EVs effects	Treatment outcomes	Ref
hUC-MSCs	Fibrotic liver	Mice model of CCl_4-induced fibrotic liver	Serum	↓ Hyaluronic acid, TGF-β1 and AST	Decreased liver damage	[212]
			Liver slices	↓ Collagen deposition		
			Liver slices	Inhibition of signaling pathway TGF-β1/Smad signaling pathway		
	Acute liver failure	Mice model of CCl_4-induced fibrotic liver	Liver slices	↓ G-CSF, IL-1α, IL-6, MCP-1 and TNF-α	Decreased liver inflammation	[266]
			Liver cell lysate	↓ IKKB/NF-κB/caspase9/3 pathway ↑ Bcl2 ↑ ERK 1/2 phosphorylation	Decreased liver cells apoptosis	
		Mice model of LPS/D-GalN-induced liver failure	Serum	↓ IL-1β and IL-6	Decreased liver inflammation	[267]
			Liver tissue homogenate	↓ NLRP3 and caspase-1 in LPS-activated macrophages		
AT-MSCs	LPS/GalN-induced fulminant hepatitis	Mice model of LPS/GalN-induced ALF	LPS-activated macrophages	↓ NLRP3 inflammasome activation	Decreased liver damage	[268]
			Serum	↓ ALT and AST		
			AT-MSC-sEVs cargo	High levels of miR-17	Modulation of inflammasome activation in hepatic macrophages	
hBM-MSCs	Fibrotic liver	Rat model of CCl_4-induced fibrotic liver	Liver homogenate	↓ Hydroxyproline	Revealed low levels of collagen deposition	[269]
			Liver homogenate	↓ MDA	Revealed low lipid peroxidation changes	
			Serum	↓ ALP, ALT, AST, GGT, total bilirubin	Decreased liver damage	
			Liver slices	↓ collagen deposition	Decrease liver damage	
			RNA extracted from liver	↓ IL- and IL-6	Reduced hepatic inflammation	
			HSCs and in liver slices	Inhibition of Wnt/β-catenin signaling	Decreased HSC activation	
hf-MSCs	Fibrotic liver	Mice model of CCl_4-induced fibrotic liver	Serum	↓ ALT and AST	Decrease liver damage	[213]
			Liver tissue	↑ expression of hepatic regenerative genes: NF-κB, cyclin D1 and cyclin E	Hepatic regeneration	
			Liver slices	$PCNA^+$-stained cells	Activation of hepatocyte proliferation	
		APAP and H_2O_2-induced liver injuries in TAMH cells	TAMH cells	↑ IL-6 and TNF-α	Initiation of liver regeneration	
				↑ iNOS and MIP-2	Hepatic regeneration	
				↑ expression of hepatic regenerative genes: Stat3 and NF-κB	Hepatic regeneration	
				↓ caspase 3 and Bcl-XL	Protection of hepatocytes from apoptosis	
hESC-MSCs	Fibrotic liver	TAA-induced chronic rat liver	Serum	↓ ALT, AST and GGT	Decrease liver damage	[270]
			Liver tissue	↓ collagen density	Decrease liver fibrosis	
				↓ caspase 3 and Bax; ↑ Bcl2	Protection of hepatocytes from apoptosis	
				↓ genes involved in liver fibrosis: Col1a, a-Sma, Timp1; ↓ pro-inflammatory genes Tnfa and Il2	Decrease liver damage	
	Drug-induced liver injury	CCl_4-induced liver injury	Liver slices	$PCNA^+$-stained cells	Activation of hepatocyte proliferation	[213]
				↑ PCNA and cyclin D1 proteins	Increase of hepatocytes survival rate	
				↑ Bcl-XL protein	Protection of hepatocytes from apoptosis	

Regarding ischemic stroke, and in response to the neural damage that follows this event, the immune system has shown to become activated. Hence, there is a rise in inflammatory mediatorś levels as well as a recruitment of immune cells for the CNS. Interestingly, several studies have shown that the administration of MSCs can reduce the levels of TNF-α and NF-κB by raising the pro-angiogenic factor VEGF. In fact, the inhibition of NF-κB is associated with anti-inflammatory and anti-apoptotic effects of MSCs. Specifically, BM-MSCs appear to stimulate brain parenchymal cells to release neurotrophic factors, including BDNF and FGF. Similarly, intravenous (*i.v.*) administration of BM-MSCs has led to segregation of the neurotrophic factors BDNF, FGF-2, HGF, IGF-1, NAP-2 and VEGF, stimulating neuronal growth and post-stroke neovascularization [17]. Importantly, as already stated for other pathological contexts, the major effects of MSCs transplantation appeared to derive from the neuroregulatory molecules that MSCs segregate, including BDNF, EGF, FGF-2 and VEGF [222,228].

In the context of multiple sclerosis (MS), MSCs have shown promising effects. Of note, MS is characterized by a chronic inflammatory demyelination in white and grey matter of the CNS, which in turn induce general brain damage. In MS, demyelination relies on oligodendrocyte damage, in part by immune cells, including T

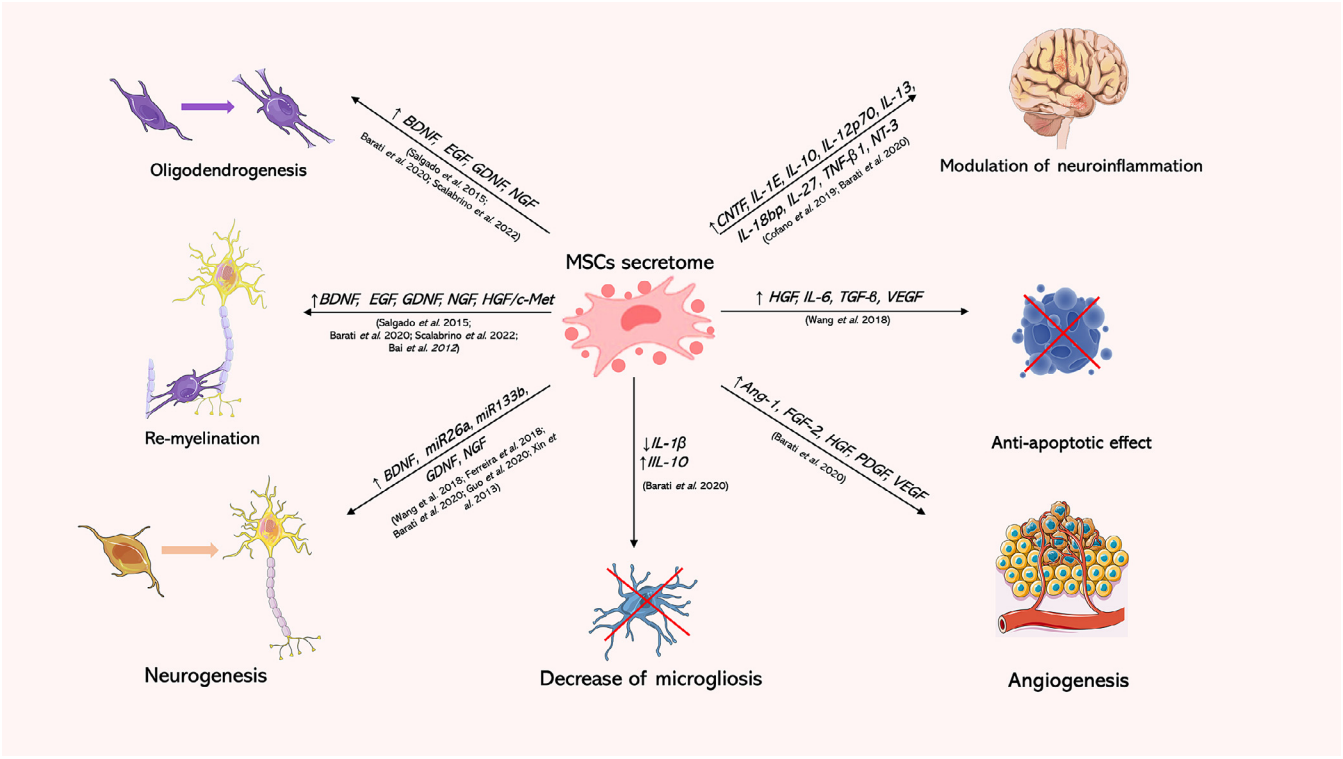


Fig. 6. The role of MSC-derived secretome in neuroregeneration. MSC release a plethora of growth factors and neurotrophins that can regulate neurogenesis, oligodendrogenesis, angiogenesis and modulates neuroinflammation, therefore contributing to neuroregeneration.
Abbreviations: Ang-1: angiopoietin 1; BDNF: brain derived growth factor; EGF: epidermal growth factor; FGF-2: fibroblast growth factor 2; CNTF: ciliary neurotrophic factor; GDNF: glial cell derived growth factor; HGF: hepatocyte growth factor; IL: interleukin; MSCs: mesenchymal stem cells; NAP-2: neutrophil activating protein 2; NGF: nerve growth factor; NT-3: neurotrophin 3; PDGF: platelet derived growth factor; TGF-β: transforming growth factor beta; TNF-β1: tumor necrosis factor beta 1; VEGF: vascular endothelial growth factor.

cells, which enter in the CNS and recognize myelin as a foreign substance to degrade it [216]. MSC-derived secretome can therefore act in distinct aspects of MS pathophysiology. For example, by *i)* stimulating oligodendrogenesis, remyelination and axonal regeneration, *ii)* inducing both anti-apoptotic signaling pathways and anti-glial scar activation, or *iii)* modulating the activity of T cells, B cells and DCs, thus controlling the autoimmune component of this disease [225]. In the experimental autoimmune encephalomyelitis (EAE), an animal model of MS, the efficacy of MSCs in enhancing functional recovery appears to reflect their ability to modulate both the immune system [229] and neural cell responses [230]. These cells have revealed to segregate several neurotrophic factors, including BDNF, neurotrophin-3 and NGF [230,231], neurotrophic factors with a pivotal role in inhibiting apoptosis and inducing the proliferation of endogenous cells in the SVZ [232–234]. In several studies, HGF, a pleiotropic cytokine derived from cells of mesenchymal origin and whose main receptor is the transmembrane c-Met [235,236], revealed a key role in mediating MSCs-stimulated recovery of autoimmune diseases, remyelination and neural cell development, including in MS [235,236]. In this regard, Bai and colleagues have identified a central role of HGF/c-Met pathway in the recovery and remyelination stimulated by MSCs. More specifically, the administration of MSC-secretome or/and HGF led to the reduction of pro-inflammatory cytokines IL-2, IL-17, IFN-γ, TNF-α, and IL-12p70 and increased the levels of anti-inflammatory cytokines IL-4 and IL-10. The use of c-Met antibody abrogated this beneficial effect [236].

Regarding Parkinsońs disease, studies with 6-hydroxydopamine (6-OHDA) animal models revealed that the administration of hBM-MSC-derived secretome significantly reduced dopaminergic loss in *substantia nigra* and *striatum*. Curiously, the same result was not observed in animals subjected to hBM-MSC engraftment, revealing that hBM-MSCs-secretome can minimize dopaminergic degeneration in a higher magnitude than BM-MSCs *per se* [237]. Importantly, it has been shown that human umbilical cord (hUC)-MSC-derived EVs pass the blood–brain barrier (BBB) and reach the midbrain *substantia nigra*. Once there, they were able to reduce dopaminergic neuron loss and improve behavioral deficits in the apomorphine-induced asymmetric rotation test in Parkinsońs disease rat models, suggesting the role of these EVs on dopaminergic neurons of the *substantia nigra* [238]. Putting in evidence the role of the oxidative stress in neurodegeneration diseases, Niu *et al.* explored the protective effect of BM-MSC-derived secretome to alleviate H_2O_2-induced oxidative stress in NSCs *in vitro*. Importantly, oxidative stress is characteristically present in SCI, leading to death of NSCs. In this regard, treatment of NSCs with H_2O_2 significantly induced apoptosis, malondialdehyde (MDA) and SOD activity by activating the Notch1 signaling pathway. BM-MSC-derived CM was shown to revert this effect, by neutralizing oxidative stress and, therefore, by preventing the apoptosis of NSCs. Notably, the survival of NSCs treated with BM-MSCS-derived CM occurred in a dose-dependent manner [126,239].

Conclusions and future perspectives

The undoubtably potential of MSC-derived secretome and the key advantages of their use over transplanting MSCs have been demonstrated by the multiple clinical trials using MSC-derived secretome, including EVs, in a wide range of human diseases (Table 6) [240]. Likewise, with the emergence of COVID-19, many efforts have been made to find new therapeutic strategies against this emerging disease. Nevertheless, despite the encouraging preclinical outcomes, described above, most of the registered clinical trials applying MSC-based therapies, for diverse human diseases,

Table 5
Preclinical studies demonstrating the beneficial effects of MSC-derived EVs on neurological diseases.
Abbreviations: BBB: blood–brain barrier; Bax: Bcl-2 associated X-protein; Bcl-2: B-cell lymphoma-2; hBM-MSCs: human bone marrow-mesenchymal stem cells; CSF: cerebrospinal fluid; EVs: extracellular vesicles; CTGF: connective tissue growth factor; LDH: lactate dehydrogenase; hUC-MSCs: human umbilical cord-mesenchymal stem cells; IL-1β: interleukin 1 beta; MCAO: middle cerebral artery occlusion; MDA: malondialdehyde; miR-133b: miRNA-133b; NSCs: neural stem cells; 6-OHDA: 6-hydroxydopamine; sEVs: small extracellular vesicles; SNL: spinal nerve ligand; SOD: superoxide dismutase; TNF-α: tumor necrosis factor alpha.

Source of EVs	Disease	Animal model	Sample	EVs effects	Treatment outcomes	Ref
rat BM-MSCs	Stroke	MCAO rat	CSF	↑ miR-133b in the ischemic cerebral tissue	Enhancement of endogenous neurogenesis and cerebral angiogenesis,synaptogenesis, neurite outgrowth and functional recovery after stroke	[16,48,120]
			Astrocytes of the ischemic boundary zone	↓ CTGF		
			Astrocytes of the ischemic boundary zone	↓ RhoA protein expression		
hUC-MSCs	Parkinsońs disease	6-OHDA-stimulated rat/6-OHDA-stimulated SH-SY5Y cells	6-OHDA-stimulated SH-SY5Y cells	↓ caspase 3	Reduction of apoptosis of dopaminergic neurons	[238]
			6-OHDA-stimulated SH-SY5Y cells	↑ autophagic-related proteins LC3B-II/I and beclin-1	Cytoprotective action	
			Striatum of 6-OHDA-stimulated rat	Upregulation of dopamine and its metabolites		
			Brain slices of 6-OHDA-stimulated rat	sEVs-crossing of BBB and reaching substantia nigra	Relieve of asymmetric rotation	
hUC-MSC	Nerve pain	SNL rat	Ipsilateral L5/L6 spinal cord and dorsal root ganglion	↓ c-Fos, CNPase, GFAP and Iba-1	Attenuated increased of glial activation	[271]
			Ipsilateral L5/L6 dorsal root ganglion	↓ TNF-α and IL-1β and ↑ IL-10, BDNF and GDNF	Anti-inflammatory and pro-neurotrophic abilities	
			Ipsilateral L5 spinal dorsal horn, dorsal root ganglion and peripheral axons	Presence of exosomes green-labelled	Homing properties of hUC-MSC-derived exosomes	
			n.a.	↑ of paw withdrawal threshold and paw withdrawal latency	↓ development of SNL-induced mechanical allodynia and thermal hyperalgesia	
hBM-MSCs	Depression	Corticosterone-induced depressed rat	Hippocampal tissue	↑ miR-26a expression	Improve injury of hippocampal neurons	[49]
			Hippocampal tissue	↓ Bax and ↑ Bcl-2	Reduction of hippocampal neurons apoptosis	
			Serum and hippocampal tissue	↑ SOD and ↓ MDA, LDH, IL-1β and TNF-α levels	Reduction of oxidate stress and inflammatory state	

have fallen short of expectations. This can be a consequence *i)* of inconsistent criteria for MSCs identity across studies, *ii)* cells inherited heterogeneity, *iii)* absence of robust good manufacturing practices (GMP)-compliant manufacturing processes, *iv)* lack of confirmation studies on safety and effectiveness through *in vitro*, *in vivo* animal models, and human clinical trials, or *v*) deficient evidence of pharmaceutical quality, stability, and shelf-life of the final product, among others.

Currently, there is no approved treatment with MSC-secretome since the safety and efficacy profiles are not sufficiently well-demonstrated yet. Therefore, there are still some challenges to overcome in what concerns to the implementation of MSC-secretome as a therapeutic with effective clinical application. Firstly, it is crucial to establish GMP guidelines for large-scale manufacturing of MSC-derived products [206,241,242]. To guarantee that GMPs are achieved, a standard protocol for both MSCs growth and production as well as for isolation of their secretome must be established to exactly define the biochemical composition of MSC-derived secretome (because it is a biological product without a well-defined chemical composition) [243]. Moreover, if a priming strategy is implemented, this must be reproducible between batches, as well as the period of the secretome conditioning [244,245]. In addition, the number of MSC passage [246–248] and the donor variability [246–248] cannot be ignored since MSC activity and, thus, the properties of MSC-secretome can be affected by donor age and sex [246,248,249], with older donors of BM-MSCs having lower proliferation rates [246,249] and osteogenic [246,248], adipogenic and neurogenic [248] capacities.

Nevertheless, there is no doubt that MSC-derived secretome can be analyzed in terms of safety, dosage and efficacy as a conventional pharmaceutical agent, offering an advantage over the use of stem cell engraftment. The clinical use of MSC-derived secretome is also facilitated by economical aspects with a favorable cost-effective profile and by easier storage procedures waiving the use of potentially toxic agents [243]. The system of secretome delivery is also a challenge since direct injection of secretome into blood circulation usually leads to a rapid uptake and clearance of the MSC-sourced secretome by macrophages [126]. Therefore, new pharmaceutical technology techniques are needed to overpass this problem, including the use of hydrogel systems to modulate secretome release. Hydrogels, for instance, can provide structural support to the therapeutic product and, on the other hand, enable a controlled release, improving the bioavailability and the safety of the therapeutic effect [126,250].

As for the use of MSC-derived EVs as therapeutic product, in a near future, it will be necessary *i)* to qualify the methods of the production, isolation, and characterization of MSC-EVs [241,251]; *ii)* define the pharmacokinetic profile of these vesicles; and *iii)* create their safety profile [251]. These requirements will be particularly important for scaling up the production of MSC-derived secretome, including MSC-EVs, since it is critical to guarantee the production of large quantities while ensuring that the produced product is similar between batches [241,242]. EVs have better safety profiles due to their better immunocompatibility, but safety and efficacy of MSC-EVs in various disease conditions need to be ensured in further preclinical and clinical evaluation. Long-term toxicity and immunogenicity of repeated administration of EVs using histopathological analysis, hematological examination, and immunotyping test should also be carried out to find whether MSC-EVs might trigger immune responses or toxic reactions

Table 6
Ongoing and completed clinical trials using MSC-derived secretome and EVs in respiratory, neurological, and other inflammatory conditions.
Abbreviations: ARDS: acute respiratory distress syndrome; AT-MSCs: adipose tissue-derived mesenchymal stem cells; BM-MSCs: bone marrow-mesenchymal stem cells; EVs: extracellular vesicles; m/lEVs: medium/large extracellular vesicles; sEVs: small extracellular vesicles; UC-MSCs: umbilical cord-derived mesenchymal stem cells.

Disease	MSC product	Administration route	n	Trial ID	Outcomes
COVID-19	MSC-secretome	IV	20	NCT05122234	Concluded. Results not published.
COVID-19	MSC-sEVs	IV	60	NCT05216562	Unknown status
COVID-19/cytokine storm	Hypoxia MSC-secretome	IM	24	NCT04753476	Unknown status
COVID-19-associated pneumonia	BM-MSC-sEVs	Inhalation	24	NCT04276987	Concluded. Results not published.
COVID-19-associated pneumonia	MSC-sEVs	Inhalation	90	NCT04602442	Concluded. Results not published.
COVID-19-associated ARDS	BM-MSC-sEVs	IV	30	NCT05125562	Withdrawn
COVID-19-associated ARDS	BM-MSC-EVs	IV infusion	400	NCT05354141	Ongoing
COVID-19-associated ARDS	BM-MSC-EVs	IV infusion	N/A	NCT04657458	Ongoing
COVID-19-associated ARDS	BM-MSC-EVs	IV infusion	120	NCT04493242	The authors reported no adverse effects.
COVID-19-associated ARDS	MSC-sEVs	IV	55	NCT04798716	Ongoing
COVID-19-associated pneumonia	MSC- sEVs	Inhalation	30	NCT04491240	The authors reported no adverse effects.
Post-acute-COVID	BM-MSC-EVs	IV	60	NCT05116761	Ongoing
ARDS	MSC- sEVs	Inhalation	169	NCT04602104	Ongoing
ARDS	BM-MSC-EVs	Injectable	81	NCT05127122	Ongoing
Bronchopulmonary dysplasia	MSC-EVs	IV infusion	3	NCT03857841	The study was interrupted due to commercial analysis and inferential analysis was not performed.
Pulmonary infection resistant to carbapenem	AT-MSC-sEVs	Inhalation	60	NCT04544215	Concluded. Necrotizing colitis was reported at the lowest dose tested.
Ischemic stroke	UC-MSC-secretome	Intranasal or intraparenchymal	15	NCT05008588	Ongoing
Cerebrovascular diseases	MSC-sEVs enriched by miR-124	Intraparenchymal	5	NCT03384433	Ongoing
Cerebral palsy	UC-MSC-secretome	Intrathecal	78	NCT04314687	Ongoing
Alzheimer's disease	MSC-sEVs	Intranasal	9	NCT04388982	Ongoing
Refractory Crohn's disease	BM-MSC-EVs	IV	N/A	NCT04388982	Ongoing
Refractory Crohn's disease	BM-MSC-EVs	IV	10	NCT05130983	Ongoing
Refractory ulcerative colitis	BM-MSC-EVs	IV	10	NCT05176366	Ongoing
Diabetes Mellitus type 1	UC-MSCs sEVS/m/lEVs	IV	20	NCT02138331	Concluded. Results not published.
Multiple organ failure	MSCs-sEVS	IV	60	NCT04356300	Ongoing

[252]. Other factors can also interfere with the EVs composition, including the number of the passage of MSCs, which can influence the EVs cargo, and the use of antibiotics, including ciprofloxacin that can alter the EVs surface, leading to their attachment to ECM components [241]. Moreover, as happens for other types of cells, the pathophysiological mechanism of MSC-EVs cannot be excluded. Since EVs enable intercellular communication, the MSC-EVs content in proteins and nucleic acids that can be involved in mechanisms of disease have to be considered and explored when trying to use these types of vesicles as a therapeutic strategy. Importantly, although the mechanisms by which EVs can cross the biological membranes and be internalized by target cells need clarification, they can bypass the BBB and the blood-retinal barrier [59]. Therefore, these EVs represent a promising therapeutic tool for several pathological conditions, namely neurological disorders [59,253–256]. Nevertheless, the risk of thrombosis cannot be ignored, especially for higher concentrations, due to the presence of procoagulant factors when systemically administered [241,257]. MSC-EVs can also be easily administrated by the intranasal route, especially for respiratory and neurological diseases. Herein, pharmaceutical technology approaches may be explored to enhance the permeability of MSC-EVs through the different biological barriers or to aid driving EVs to target organs. These methods include the application of excipients and engineering techniques that can improve MSC-EVs their permeability and structure, such as increasing the expression of surface proteins that can target them into a certain tissue or that can prevent their recognition by immune cells, including CD47 to avoid phagocytosis by monocytes, thereby prolonging the circulation half-life of EVs [258,259]. In fact, some clinical trials with MSC-secretome already include non-invasive routes of administration such as the intranasal route for Alzheimeŕs disease (NCT04388982) and for acute ischemic stroke (NCT05008588) (Table 6). Additionally, in order to overcome existing obstacles in EVs-based therapeutics, it is imperative to standardize and optimize the production of EVs, while also better understanding their underlying mechanisms.

Lastly, the precise mechanisms by which MSCs exert their trophic and therapeutic effects in different biological contexts still needs clarification. Additional molecular and biochemical studies are required to fill the gap in our understanding on the exact signaling pathways responsible for the strong association between MSC secretome and recipient cell survival and tissue regeneration. Nevertheless, the preclinical studies together with more standardized clinical trials are developing rapidly which may provide critical guidance for researcher pursuing further translational processes. All in all, the following years will be certainly determinant to overcome the announced critical challenges of MSC-derived secretome, making its clinical application more suitable for the most diverse areas of regenerative medicine.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work was financially supported by Fundação para a Ciência e a Tecnologia, Portugal through SFRH/BD/09328/2021 to Trigo; SFRH/BD/144130/2019 to Rodrigues; UIDB/04138/2020; UIDP/04138/2020. This project has received funding from the European Horizon's research and innovation programme HORIZON-HLTH-2022-STAYHLTH-02 under agreement No 101095679.

References

[1] Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Prolif 1970;3:393–403. doi: https://doi.org/10.1111/j.1365-2184.1970.tb00347.x.

[2] Tullis GE, Spears K, Kirk MD. Immunological barriers to stem cell therapy in the central nervous system. Stem Cells Int 2014;2014:. doi: https://doi.org/10.1155/2014/507905507905.

[3] Farfán N, Carril J, Redel M, Zamorano M, Araya M, Monzón E, et al. Intranasal administration of mesenchymal stem cell secretome reduces hippocampal oxidative stress, neuroinflammation and cell death, improving the behavioral outcome following perinatal asphyxia. Int J Mol Sci 2020;21:. doi: https://doi.org/10.3390/ijms21207800 7800.

[4] Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. Npj Regene Med 2019;4(1):1–15. doi: 10.1038/s41536-019-0083-6.

[5] Dexter TM, Spooncer E. Growth and differentiation in the hemopoietic system. Annu Rev Cell Biol 1987;3:423–41. doi: https://doi.org/10.1146/annurev.cb.03.110187.002231.

[6] Dexter TM, Spooncer E, Toksoz D, Lajtha LG. The role of cells and their products in the regulation of in vitro stem cell proliferation and granulocyte development. J Supramol Struct 1980;13:513–24. doi: https://doi.org/10.1002/JSS.400130410.

[7] Noronha Nc NDC, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, et al. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. Stem Cell Res Ther 2019;10. doi: https://doi.org/10.1186/s13287-019-1224-y.

[8] Miranda JP, Camões SP, Gaspar MM, Rodrigues JS, Carvalheiro M, Bárcia RN, et al. The secretome derived from 3D-cultured umbilical cord tissue MSCs counteracts manifestations typifying rheumatoid arthritis. Front Immunol 2019;10:. doi: https://doi.org/10.3389/FIMMU.2019.0001818.

[9] Camões SP, Bulut O, Yazar V, Gaspar MM, Simões S, Ferreira R, et al. 3D-MSCs A151 ODN-loaded exosomes are immunomodulatory and reveal a proteomic cargo that sustains wound resolution. J Adv Res 2022. doi: https://doi.org/10.1016/j.jare.2022.01.013.

[10] Marques CR, Marote A, Mendes-Pinheiro B, Teixeira FG, Salgado AJ. Cell secretome based approaches in Parkinson's disease regenerative medicine. Expert Opin Biol Ther 2018;18:1235–45. doi: https://doi.org/10.1080/14712598.2018.1546840.

[11] Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell 2011;9:11–5. doi: https://doi.org/10.1016/J.STEM.2011.06.008.

[12] Bárcia RN, Santos JM, Teixeira M, Filipe M, Pereira ARS, Ministro A, et al. Umbilical cord tissue–derived mesenchymal stromal cells maintain immunomodulatory and angiogenic potencies after cryopreservation and subsequent thawing. Cytotherapy 2017;19:360–70. doi: https://doi.org/10.1016/j.jcyt.2016.11.008.

[13] Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013;45:e54. doi: 10.1038/emm.2013.94.

[14] Conforti A, Scarsella M, Starc N, Giorda E, Biagini S, Proia A, et al. Microvescicles derived from mesenchymal stromal cells are not as effective as their cellular counterpart in the ability to modulate immune responses in vitro. Stem Cells Dev 2014;23:. doi: https://doi.org/10.1089/SCD.2014.0091 2591.

[15] Mancuso P, Raman S, Glynn A, Barry F, Murphy JM. Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome. Front Bioeng Biotechnol 2019;7:. doi: https://doi.org/10.3389/fbioe.2019.000099.

[16] Xin H, Li Y, Chopp M. Exosomes/miRNAs as mediating cell-based therapy of stroke. Front Cell Neurosci 2014;8. doi: https://doi.org/10.3389/fncel.2014.00377.

[17] Wang F, Tang H, Zhu J, Zhang JH. Transplanting mesenchymal stem cells for treatment of ischemic stroke. Cell Transplant 2018;27:1825–34. doi: https://doi.org/10.1177/0963689718795424.

[18] Cunningham CJ, Redondo-Castro E, Allan SM. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. J Cerebral Blood Flow Metab 2018;38:1276–92. doi: https://doi.org/10.1177/0271678X18776802.

[19] The safety and the efficacy evaluation of allogenic adipose MSC-exos in patients with Alzheimer's disease. National Library of Medicine (US) Identifier: NCT04388982 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT04388982 (accessed July 13, 2023).

[20] A clinical study of mesenchymal stem cell exosomes nebulizer for the treatment of ARDS. National Library of Medicine (US) Identifier: NCT04602104 n.d. https://classic.clinicaltrials.gov/ct2/show/results/NCT04602104 (accessed July 13, 2023).

[21] Bone marrow mesenchymal stem cell derived extracellular vesicles infusion treatment for ARDS (EXIT-ARDS). National Library of Medicine (US) Identifier: NCT05127122 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT05127122 (accessed July 13, 2023).

[22] A safety study of IV stem cell-derived extracellular vesicles (UNEX-42) in preterm neonates at high risk for BPD. National Library of Medicine (US) Identifier: NCT03857841 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT03857841 (accessed July 13, 2023).

[23] Fröhlich E. Therapeutic potential of mesenchymal stem cells and their products in lung diseases—intravenous administration versus inhalation. Pharmaceutics 2021;13:1–22. doi: https://doi.org/10.3390/pharmaceutics13020232.

[24] Chouw A, Milanda T, Sartika CR, Kirana MN, Halim D, Faried A. Potency of mesenchymal stem cell and its secretome in treating COVID-19. Regen Eng Transl Med 2021:1–12. doi: https://doi.org/10.1007/s40883-021-00202-5.

[25] Mesenchymal stem cell secretome in severe cases of COVID-19. National Library of Medicine (US) Identifier: NCT05122234 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT05122234 (accessed July 13, 2023).

[26] Efficacy and safety of EXOSOME-MSC therapy to reduce hyper-inflammation in moderate COVID-19 patients (EXOMSC-COV19). National Library of Medicine (US) Identifier: NCT05216562 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT05216562 (accessed July 13, 2023).

[27] Treatment of severe COVID-19 patients using secretome of hypoxia-mesenchymal stem cells in Indonesia. National Library of Medicine (US) Identifier: NCT04753476 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT04753476 (accessed July 13, 2023).

[28] A pilot clinical study on inhalation of mesenchymal stem cells exosomes treating severe novel coronavirus pneumonia. National Library of Medicine (US) Identifier: NCT04276987 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT04276987 (accessed July 13, 2023).

[29] Bone marrow mesenchymal stem cell derived extracellular vesicles infusion treatment for mild-to-moderate COVID-19: a phase II clinical trial. National Library of Medicine (US) Identifier: NCT05125562 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT05125562 (accessed July 13, 2023).

[30] Extracellular vesicle treatment for acute respiratory distress syndrome (ARDS) (EXTINGUISH ARDS). National Library of Medicine (US) Identifier: NCT05354141 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT05354141 (accessed July 13, 2023).

[31] Extracellular vesicle infusion treatment for COVID-19 associated ARDS (EXIT-COVID19). National Library of Medicine (US) Identifier: NCT04493242 n.d. https://classic.clinicaltrials.gov/ct2/show/NCT04493242 (accessed July 19, 2023).

[32] Rohban R, Pieber TR. Mesenchymal stem and progenitor cells in regeneration: tissue specificity and regenerative potential. Stem Cells Int 2017;2017. doi: https://doi.org/10.1155/2017/5173732.

[33] Berebichez-Fridman R, Montero-Olvera PR. Sources and clinical applications of mesenchymal stem cells: state-of-the-art review. Sultan Qaboos Univ Med J 2018;18:e264–77. doi: https://doi.org/10.18295/SQUMJ.2018.18.03.002.

[34] da Silva ML, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006;119:2204–13. doi: https://doi.org/10.1242/JCS.02932.

[35] Singh A, Singh A, Sen D. Mesenchymal stem cells in cardiac regeneration: a detailed progress report of the last 6 years (2010–2015). Stem Cell Res Ther 2016;7. doi: 10.1186/s13287-016-0341-0.

[36] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–7. doi: https://doi.org/10.1080/14653240600855905.

[37] Zhang G-L, Zhu Z-H, Wang Y-Z. Neural stem cell transplantation therapy for brain ischemic stroke: review and perspectives. World J Stem Cells 2019;11:817–30. doi: https://doi.org/10.4252/wjsc.v11.i10.817.

[38] Camões SP, Santos JM, Carvalho F, Miranda JP. Mesenchymal stem cells for cutaneous wound healing. In: Rodrigues G, Roelen BAJ, editors. Concepts and applications of stem cell biology, learning materials in biosciences. Cham: Springer; 2020. p. 247–67. doi: https://doi.org/10.1007/978-3-030-43939-2_13.

[39] Santos JMM, Camões SPP, Filipe E, Cipriano M, Barcia RNN, Filipe M, et al. Three-dimensional spheroid cell culture of umbilical cord tissue-derived mesenchymal stromal cells leads to enhanced paracrine induction of wound healing. Stem Cell Res Ther 2015;6:90. doi: https://doi.org/10.1186/s13287-015-0082-5.

[40] Miranda JP, Filipe E, Fernandes AS, Almeida JM, Martins JP, De La Fuente A, et al. The human umbilical cord tissue-derived MSC population UCX ® promotes early motogenic effects on keratinocytes and fibroblasts and G-CSF-mediated mobilization of BM-MSCs when transplanted in vivo. Cell Transplant 2015;24:865–77. doi: https://doi.org/10.3727/096368913X676231.

[41] Lai RC, Chen TS, Lim SK. Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease. Regener Med 2011;6:481–92. doi: https://doi.org/10.2217/RME.11.35.

[42] Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci 2017;74:2345–60. doi: https://doi.org/10.1007/s00018-017-2473-5.

[43] Serras AS, Camões SP, Antunes B, Costa VM, Dionísio F, Yazar V, et al. The secretome of human neonatal mesenchymal stem cells modulates doxorubicin-induced cytotoxicity: impact in non-tumor cells. Int J Mol Sci 2021;22. doi: https://doi.org/10.3390/ijms222313072.

[44] Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Res Ther 2016;7:1–13. doi: 10.1186/S13287-016-0363-7.

[45] Vassileff N, Cheng L, Hill AF. Extracellular vesicles - Propagators of neuropathology and sources of potential biomarkers and therapeutics for neurodegenerative diseases. J Cell Sci 2020;133. doi: https://doi.org/10.1242/JCS.243139/226235.

[46] Ferguson SW, Wang J, Lee CJ, Liu M, Neelamegham S, Canty JM, et al. The microRNA regulatory landscape of MSC-derived exosomes: a systems view. Sci Rep 2018;8:1–12. doi: 10.1038/s41598-018-19581-x.

[47] Reis M, Mavin E, Nicholson L, Green K, Dickinson AM, Wang XN. Mesenchymal stromal cell-derived extracellular vesicles attenuate dendritic cell maturation and function. Front Immunol 2018;9:. doi: https://doi.org/10.3389/FIMMU.2018.02538/FULL2538.

[48] Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, et al. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. Stem Cells 2013;31. doi: https://doi.org/10.1002/stem.1409.

[49] Guo H, Huang B, Wang Y, Zhang Y, Ma Q, Ren Y. Bone marrow mesenchymal stem cells-derived exosomes improve injury of hippocampal neurons in rats with depression by upregulating microRNA-26a expression. Int Immunopharmacol 2020;82:. doi: https://doi.org/10.1016/J.INTIMP.2020.106285106285.

[50] Garcia G, Fernandes A, Stein F, Brites D. Protective signature of IFNγ-stimulated microglia relies on miR-124-3p regulation from the secretome released by mutant APP Swedish neuronal cells. Front Pharmacol 2022;13. doi: https://doi.org/10.3389/fphar.2022.833066.

[51] Blazquez R, Sanchez-Margallo FM, de la Rosa O, Dalemans W, Álvarez V, Tarazona R, et al. Immunomodulatory potential of human adipose mesenchymal stem cells derived exosomes on in vitro stimulated T cells. Front Immunol 2014;5:. doi: https://doi.org/10.3389/FIMMU.2014.00556/ABSTRACT96446.

[52] Franco da Cunha F, Andrade-Oliveira V, Candido de Almeida D, Borges da Silva T, Naffah de Souza Breda C, Costa Cruz M, et al. Extracellular vesicles isolated from mesenchymal stromal cells modulate CD4+ T lymphocytes toward a regulatory profile. Cells 2020;9:1059. doi: 10.3390/CELLS9041059.

[53] Sdrimas K, Kourembanas S. MSC microvesicles for the treatment of lung disease: a new paradigm for cell-free therapy. Antioxid Redox Signal 2014;21:1905–15. doi: https://doi.org/10.1089/ars.2013.5784.

[54] Pinho AG, Cibrão JR, Silva NA, Monteiro S, Salgado AJ. Cell secretome: basic insights and therapeutic opportunities for CNS disorders. Pharmaceuticals 2020;13:. doi: https://doi.org/10.3390/ph1302003131.

[55] Papait A, Ragni E, Cargnoni A, Vertua E, Romele P, Masserdotti A, et al. Comparison of EV-free fraction, EVs, and total secretome of amniotic mesenchymal stromal cells for their immunomodulatory potential: a translational perspective. Front Immunol 2022;13:. doi: https://doi.org/10.3389/FIMMU.2022.960909/BIBTEX960909.

[56] Del FA, Luciano R, Pascucci L, Goffredo BM, Giorda E, Scapaticci M, et al. Immunoregulatory effects of mesenchymal stem cell-derived extracellular vesicles on T lymphocytes. Cell Transplant 2015;24:2615–27. doi: https://doi.org/10.3727/096368915X687543.

[57] Gouveia De Andrade AV, Bertolino G, Riewaldt J, Bieback K, Karbanová J, Odendahl M, et al. Extracellular vesicles secreted by bone marrow- and adipose tissue-derived mesenchymal stromal cells fail to suppress lymphocyte proliferation. Stem Cells Dev 2015;24:1374–6. doi: https://doi.org/10.1089/SCD.2014.0563.

[58] Eleuteri S, Fierabracci A. Insights into the secretome of mesenchymal stem cells and its potential applications. Int J Mol Sci 2019;20:. doi: https://doi.org/10.3390/ijms20184597.

[59] Ping JYX, Neupane YR, Pastorin G, Ping JYX, Neupane YR, Pastorin G. Extracellular vesicles and their interplay with biological membranes 2021. doi: 10.5772/INTECHOPEN.101297.

[60] Song N, Wakimoto H, Rossignoli F, Bhere D, Ciccocioppo R, Chen KS, et al. Mesenchymal stem cell immunomodulation: in pursuit of controlling COVID-19 related cytokine storm. Stem Cells 2021;39:707–22. doi: https://doi.org/10.1002/STEM.3354.

[61] Saeedi P, Halabian R, Fooladi AAI. A revealing review of mesenchymal stem cells therapy, clinical perspectives and Modification strategies. Stem Cell Investig 2019:6. doi: https://doi.org/10.21037/SCI.2019.08.11.

[62] Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 2009;5:54. doi: https://doi.org/10.1016/J.STEM.2009.05.003.

[63] Zhou Y, Yamamoto Y, Xiao Z, Ochiya T. The immunomodulatory functions of mesenchymal stromal/stem cells mediated via paracrine activity. J Clin Med 2019:8. doi: https://doi.org/10.3390/JCM8071025.

[64] Brandau S, Jakob M, Hemeda H, Bruderek K, Janeschik S, Bootz F, et al. Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge. J Leukoc Biol 2010;88:1005–15. doi: https://doi.org/10.1189/JLB.0410207.

[65] Jiang D, Muschhammer J, Qi Y, Kügler A, de Vries JC, Saffarzadeh M, et al. Suppression of neutrophil-mediated tissue damage—a novel skill of mesenchymal stem cells. Stem Cells 2016;34:2393–406. doi: https://doi.org/10.1002/STEM.2417.

[66] Liu Y, Zeng R, Wang Y, Huang W, Hu B, Zhu G, et al. Mesenchymal stem cells enhance microglia M2 polarization and attenuate neuroinflammation through TSG-6. Brain Res 2019;1724:. doi: https://doi.org/10.1016/J.BRAINRES.2019.146422146422.

[67] Day AJ, Milner CM. TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol 2019;78–79:60–83. doi: https://doi.org/10.1016/J.MATBIO.2018.01.011.

[68] Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, et al. Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. Stem Cells 2014;32:116–25. doi: https://doi.org/10.1002/stem.1504.

[69] Rajan TS, Giacoppo S, Diomede F, Ballerini P, Paolantonio M, Marchisio M, et al. The secretome of periodontal ligament stem cells from MS patients protects against EAE. Sci Rep 2016:6. doi: https://doi.org/10.1038/SREP38743.

[70] Németh K, Leelahavanichkul A, Yuen PST, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 2009;15:42–9. doi: https://doi.org/10.1038/NM.1905.

[71] Yang R, Gao H, Chen L, Fang N, Chen H, Song G, et al. Effect of peripheral blood-derived mesenchymal stem cells on macrophage polarization and Th17/Treg balance in vitro. Regen Ther 2020;14:275–83. doi: https://doi.org/10.1016/J.RETH.2020.03.008.

[72] Thomas H, Jäger M, Mauel K, Brandau S, Lask S, Flohé SB. Interaction with mesenchymal stem cells provokes natural killer cells for enhanced IL-12/IL-18-induced interferon-gamma secretion. Mediat Inflamm 2014; 2014.. doi: https://doi.org/10.1155/2014/143463.

[73] Prockop DJ, Youn OJ. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. Mol Ther 2012;20:14–20. doi: https://doi.org/10.1038/mt.2011.211.

[74] Harrell CR, Markovic BS, Fellabaum C, Arsenijevic N, Djonov V, Volarevic V. The role of Interleukin 1 receptor antagonist in mesenchymal stem cell-based tissue repair and regeneration. BioFactors 2020;46:263–75. doi: https://doi.org/10.1002/BIOF.1587.

[75] Hyvärinen K, Holopainen M, Skirdenko V, Ruhanen H, Lehenkari P, Korhonen M, et al. Mesenchymal stromal cells and their extracellular vesicles enhance the anti-inflammatory phenotype of regulatory macrophages by downregulating the production of interleukin (IL)-23 and IL-22. Front Immunol 2018;9:. doi: https://doi.org/10.3389/FIMMU.2018.00771/BIBTEX771.

[76] Liu Y, Yin Z, Zhang R, Yan K, Chen L, Chen F, et al. MSCs inhibit bone marrow-derived DC maturation and function through the release of TSG-6. Biochem Biophys Res Commun 2014;450:1409–15. doi: https://doi.org/10.1016/J.BBRC.2014.07.001.

[77] Newton K, Dixit VM. Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol 2012:4. doi: https://doi.org/10.1101/cshperspect.a006049.

[78] Manzoor Z, Koh YS. Mitogen-activated protein kinases in inflammation. J Bacteriol Virol 2012:42. doi: https://doi.org/10.4167/jbv.2012.42.3.189.

[79] Lee K, Park N, Jung H, Rim YA, Nam Y, Lee J, et al. Mesenchymal stem cells ameliorate experimental arthritis via expression of interleukin-1 receptor antagonist. PLoS One 2018:13. doi: https://doi.org/10.1371/JOURNAL.PONE.0193086.

[80] Lu Z, Chang W, Meng S, Xu X, Xie J, Guo F, et al. Mesenchymal stem cells induce dendritic cell immune tolerance via paracrine hepatocyte growth factor to alleviate acute lung injury. Stem Cell Res Ther 2019;10:1–16. doi: https://doi.org/10.1186/S13287-019-1488-2/FIGURES/6.

[81] Li H, Guo Z, Jiang X, Zhu H, Li X, Mao N. Mesenchymal stem cells alter migratory property of T and dendritic cells to delay the development of murine lethal acute graft-versus-host disease. Stem Cells 2008;26:2531–41. doi: https://doi.org/10.1634/STEMCELLS.2008-0146.

[82] Huang Y, Chen P, Zhang CB, Ko GJ, Ruiz M, Fiorina P, et al. Kidney-derived mesenchymal stromal cells modulate dendritic cell function to suppress alloimmune responses and delay allograft rejection. Transplantation 2010;90:1307–11. doi: https://doi.org/10.1097/TP.0B013E3181FDD9EB.

[83] Moloudizargari M, Govahi A, Fallah M, Rezvanfar MA, Asghari MH, Abdollahi M. The mechanisms of cellular crosstalk between mesenchymal stem cells and natural killer cells: therapeutic implications. J Cell Physiol 2021;236:2413–29. doi: https://doi.org/10.1002/JCP.30038.

[84] Chatterjee D, Marquardt N, Tufa DM, Hatlapatka T, Hass R, Kasper C, et al. Human umbilical cord-derived mesenchymal stem cells utilise activin-A to suppress interferon-gamma production by natural killer cells. Front Immunol 2014;5:662. doi: https://doi.org/10.1186/s13287-021-02222-y.

[85] Shi Y, Su J, Roberts AI, Shou P, Rabson AB, Ren G. How mesenchymal stem cells interact with tissue immune responses. Trends Immunol 2012;33:136. doi: https://doi.org/10.1016/J.IT.2011.11.004.

[86] Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006;24:74–85. doi: https://doi.org/10.1634/STEMCELLS.2004-0359.
[87] Lee S, Kim S, Chung H, Moon JH, Kang SJ, Park CG. Mesenchymal stem cell-derived exosomes suppress proliferation of T cells by inducing cell cycle arrest through p27kip1/Cdk2 signaling. Immunol Lett 2020;225:16–22. doi: https://doi.org/10.1016/J.IMLET.2020.06.006.
[88] Müller L, Tunger A, Wobus M, von Bonin M, Towers R, Bornhäuser M, et al. Immunomodulatory properties of mesenchymal stromal cells: an update. Front Cell Dev Biol 2021;9:179. doi: https://doi.org/10.3389/fcell.2021.637725.
[89] Li X, Xu Z, Bai J, Yang S, Zhao S, Zhang Y, et al. Umbilical cord tissue-derived mesenchymal stem cells induce T lymphocyte apoptosis and cell cycle arrest by expression of indoleamine 2, 3-dioxygenase. Stem Cells Int 2016;2016. doi: https://doi.org/10.1155/2016/7495135.
[90] Hee PH, Lee S, Yu Y, Yoo SM, Baek SY, Jung N, et al. TGF-β secreted by human umbilical cord blood-derived mesenchymal stem cells ameliorates atopic dermatitis by inhibiting secretion of TNF-α and IgE. Stem Cells 2020;38:904–16. doi: https://doi.org/10.1002/STEM.3183.
[91] Luz-Crawford P, Djouad F, Toupet K, Bony C, Franquesa M, Hoogduijn MJ, et al. Mesenchymal stem cell-derived interleukin 1 receptor antagonist promotes macrophage polarization and inhibits B cell differentiation. Stem Cells 2016;34:483–92. doi: https://doi.org/10.1002/STEM.2254.
[92] Magatti M, Masserdotti A, Bonassi Signoroni P, Vertua E, Stefani FR, Silini AR, et al. B lymphocytes as targets of the immunomodulatory properties of human amniotic mesenchymal stromal cells. Front Immunol 2020;11:. doi: https://doi.org/10.3389/FIMMU.2020.01156/BIBTEX1156.
[93] Bogdan C. Nitric oxide and the immune response. Nat Immunol 2001;2:907–16. doi: https://doi.org/10.1038/NI1001-907.
[94] Bari E, Ferrarotti I, Di Silvestre D, Grisoli P, Barzon V, Balderacchi A, et al. Adipose mesenchymal extracellular vesicles as Alpha-1-antitrypsin physiological delivery systems for lung regeneration. Cells 2019;8:1–18. doi: https://doi.org/10.3390/cells8090965.
[95] Bar JK, Lis-Nawara A, Grelewski PG. Dental pulp stem cell-derived secretome and its regenerative potential. Int J Mol Sci 2021:22. doi: https://doi.org/10.3390/ijms222112018.
[96] Burlacu A, Grigorescu G, Rosca AM, Preda MB, Simionescu M. Factors secreted by mesenchymal stem cells and endothelial progenitor cells have complementary effects on angiogenesis in vitro. Stem Cells Dev 2013:22. doi: https://doi.org/10.1089/scd.2012.0273.
[97] Ling YQ, Zhang YG, Chen Q. Mesenchymal stem cell (MSC)-derived extracellular vesicles: potential therapeutics as MSC trophic mediators in regenerative medicine. Anatom Rec 2020:303. doi: https://doi.org/10.1002/ar.24186.
[98] Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. J Cerebral Blood Flow Metab 2013:33. doi: https://doi.org/10.1038/jcbfm.2013.152.
[99] Takeuchi R, Katagiri W, Endo S, Kobayashi T. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. PLoS One 2019:. doi: https://doi.org/10.1371/journal.pone.022547214.
[100] Katsuda T, Tsuchiya R, Kosaka N, Yoshioka Y, Takagaki K, Oki K, et al. Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. Sci Rep 2013:3. doi: https://doi.org/10.1038/srep01197.
[101] Khan M, Adil SER, Olson AL. The role of mesenchymal stem cells in oncology and regenerative medicine. Fut Oncol 2017;13:821–31. doi: https://doi.org/10.2217/fon-2016-0264.
[102] Jotzu C, Eckhard A, Welte G, Li J, Hennessy BT, Devarajan E, et al. Adipose tissue-derived stem cells differentiate into carcinoma-associated fibroblast-like cells under the influence of tumor-derived factors. Anal Cell Pathol (Amst) 2010;33:61–79. doi: https://doi.org/10.3233/ACP-CLO-2010-0535.
[103] Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Kodama M, Higashi Y, et al. Mesenchymal stem cells enhance growth and metastasis of colon cancer. Int J Cancer 2010;127:2323–33. doi: https://doi.org/10.1002/IJC.25440.
[104] Chang F, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, et al. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. Leukemia 2003;17:590–603. doi: https://doi.org/10.1038/sj.leu.2402824.
[105] Meleshina AV, Cherkasova EI, Shirmanova MV, Klementieva NV, Kiseleva EV, Snopova LB, et al. Influence of mesenchymal stem cells on metastasis development in mice in vivo. Stem Cell Res Ther 2015;6:1–10.
[106] Gomes ED, Vieira de Castro J, Costa BM, Salgado AJ. The impact of Mesenchymal Stem Cells and their secretome as a treatment for gliomas. Biochimie 2018;155. doi: https://doi.org/10.1016/j.biochi.2018.07.008.
[107] Sousa A, Coelho P, Leite F, Teixeira C, Rocha AC, Santos I, et al. Impact of umbilical cord mesenchymal stromal/stem cell secretome and cord blood serum in prostate cancer progression. Hum Cell 2023;36. doi: https://doi.org/10.1007/s13577-023-00880-z.
[108] Lu Y, Yuan Y, Wang X, Wei L, Chen Y, Cong C, et al. The growth inhibitory effect of mesenchymal stem cells on tumor cells in vitro and in vivo. Cancer Biol Ther 2008;4047. doi: https://doi.org/10.4161/cbt.7.2.5296.
[109] Dasari VR, Velpula KK, Kaur K, Fassett D, Klopfenstein JD, Dinh DH, et al. Cord blood stem cell-mediated induction of apoptosis in glioma downregulates X-linked inhibitor of apoptosis protein (XIAP). PLoS One 2010;5. doi: 10.1371/journal.pone.0011813.
[110] Ramasamy R, Lam E-W-F, Soeiro I, Tisato V, Bonnet D, Dazzi F. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on in vivo tumor growth. Leukemia: Off J Leukemia Soc Am, Leukemia Res Fund, UK 2007;21:304–10. doi: https://doi.org/10.1038/sj.leu.2404489.
[111] Gauthaman K, Yee FC, Cheyyatraivendran S, Biswas A, Choolani M, Bongso A. Human umbilical cord wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growth in vitro. J Cell Biochem 2012;113:2027–39. doi: https://doi.org/10.1002/jcb.24073.
[112] Gauthaman K, Fong CY, Arularasu S, Subramanian A, Biswas A, Choolani M, et al. Human Wharton's jelly stem cell conditioned medium and cell-free lysate inhibit human osteosarcoma and mammary carcinoma cell growth in vitro and in xenograft mice. J Cell Biochem 2013;114:366–77. doi: https://doi.org/10.1002/jcb.24367.
[113] Ma Y, Hao X, Zhang S, Zhang J. The in vitro and in vivo effects of human umbilical cord mesenchymal stem cells on the growth of breast cancer cells. Breast Cancer Res Treat 2012;133:473–85. doi: https://doi.org/10.1007/s10549-011-1774-x.
[114] Qiao L, Xu Z-L, Zhao T-J, Ye L-H, Zhang X-D. Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling. Cancer Lett 2008;269:67–77.
[115] Dasari VR, Kaur K, Velpula KK, Gujrati M, Fassett D, Klopfenstein JD, et al. Up regulation of PTEN in glioma cells by cord blood mesenchymal stem cells inhibits migration via downregulation of the PI3K/Akt pathway. PLoS One 2010;5. doi: https://doi.org/10.1371/journal.pone.0010350.
[116] Qiao L, Xu Z, Zhao T, Zhao Z, Shi M, Zhao RC, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. Cell Res 2008;18:500–7. doi: https://doi.org/10.1038/cr.2008.40\rcr200840 [pii].
[117] Zhu Y, Sun Z, Han Q, Liao L, Wang J, Bian C, et al. Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1. Leukemia: Off J Leukemia Soc Am, Leukemia Res Fund, UK 2009;23:925–33. doi: https://doi.org/10.1038/leu.2008.384.
[118] Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. Nat Rev Genet 2004;5:691–701. doi: https://doi.org/10.1038/nrg1427.
[119] Eiro N, Sendon-Lago J, Seoane S, Bermudez MA, Lamelas ML, Garcia-Caballero T, et al. Potential therapeutic effect of the secretome from human uterine cervical stem cells against both cancer and stromal cells compared with adipose tissue stem cells. Oncotarget 2014;5:10692–708.
[120] Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Gonçalves RM. Mesenchymal stromal cell secretome: influencing therapeutic potential by cellular pre-conditioning. Front Immunol 2018;9:. doi: https://doi.org/10.3389/fimmu.2018.028372837.
[121] Ozkan S, Isildar B, Ercin M, Gezginci-Oktayoglu S, Konukoglu D, Neşetoğlu N, et al. Therapeutic potential of conditioned medium obtained from deferoxamine preconditioned umbilical cord mesenchymal stem cells on diabetic nephropathy model. Stem Cell Res Ther 2022;13. doi: 10.1186/s13287-022-03121-6.
[122] Antebi B, Rodriguez LA, Walker KP, Asher AM, Kamucheka RM, Alvarado L, et al. Short-term physiological hypoxia potentiates the therapeutic function of mesenchymal stem cells. Stem Cell Res Ther 2018;9:1–15. doi: 10.1186/S13287-018-1007-X.
[123] Chen L, Xu Y, Zhao J, Zhang Z, Yang R, Xie J, et al. Conditioned medium from hypoxic bone marrow-derived mesenchymal stem cells enhances wound healing in mice. PLoS One 2014;9. doi: 10.1371/journal.pone.0096161.
[124] Han KH, Kim AK, Kim MH, Kim DH, Go HN, Kim DI. Enhancement of angiogenic effects by hypoxia-preconditioned human umbilical cord-derived mesenchymal stem cells in a mouse model of hindlimb ischemia. Cell Biol Int 2016;40:27–35. doi: https://doi.org/10.1002/CBIN.10519.
[125] Yu J, Yin S, Zhang W, Gao F, Liu Y, Chen Z, et al. Hypoxia preconditioned bone marrow mesenchymal stem cells promote liver regeneration in a rat massive hepatectomy model. Stem Cell Res Ther 2013;4:83. doi: https://doi.org/10.1186/SCRT234.
[126] Gwam C, Mohammed N, Ma X. Stem cell secretome, regeneration, and clinical translation: a narrative review. Ann Transl Med 2021;9:70. doi: https://doi.org/10.21037/ATM-20-5030.
[127] Hong H-E, Kim O-H, Kwak BJ, Choi HJ, Im K-H, Ahn J, et al. Antioxidant action of hypoxic conditioned media from adipose-derived stem cells in the hepatic injury of expressing higher reactive oxygen species. Ann Surg Treat Res 2019;97:. doi: https://doi.org/10.4174/ASTR.2019.97.4.159159.
[128] Teixeira FG, Panchalingam KM, Anjo SI, Manadas B, Pereira R, Sousa N, et al. Do hypoxia/normoxia culturing conditions change the neuroregulatory profile of Wharton Jelly mesenchymal stem cell secretome? Stem Cell Res Ther 2015;6. doi: 10.1186/s13287-015-0124-z.
[129] Cicione C, Muiños-López E, Hermida-Gómez T, Fuentes-Boquete I, Díaz-Prado S, Blanco FJ. Effects of severe hypoxia on bone marrow mesenchymal stem cells differentiation potential. Stem Cells Int 2013;2013. doi: https://doi.org/10.1155/2013/232896.
[130] Lavrentieva A, Majore I, Kasper C, Hass R. Effects of hypoxic culture conditions on umbilical cord-derived human mesenchymal stem cells. Cell Commun Signal 2010;8:. doi: https://doi.org/10.1186/1478-811X-8-1818.
[131] Man K, Brunet MY, Lees R, Peacock B, Cox SC. Epigenetic reprogramming via synergistic hypomethylation and hypoxia enhances the therapeutic efficacy of mesenchymal stem cell extracellular vesicles for bone repair. Int J Mol Sci 2023;24:. doi: https://doi.org/10.3390/IJMS24087564/S17564.

[132] Li M, Jiang Y, Hou Q, Zhao Y, Zhong L, Fu X. Potential pre-activation strategies for improving therapeutic efficacy of mesenchymal stem cells: current status and future prospects. Stem Cell Res Ther 2022;13:1–21. doi: 10.1186/S13287-022-02822-2.
[133] Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014;15:1009–16. doi: https://doi.org/10.1038/ni.3002.
[134] Noronha N de C, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, et al. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. Stem Cell Res Ther 2019;10:1–21. doi: 10.1186/S13287-019-1224-Y.
[135] Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2:141–50. doi: https://doi.org/10.1016/J.STEM.2007.11.014.
[136] Chinnadurai R, Copland IB, Patel SR, Galipeau J. IDO-independent suppression of T cell effector function by IFN-γ–licensed human mesenchymal stromal cells. J Immunol 2014;192:1491–501. doi: https://doi.org/10.4049/jimmunol.1301828.
[137] Chen H, Min XH, Wang QY, Leung FW, Shi L, Zhou Y, et al. Pre-activation of mesenchymal stem cells with TNF-α, IL-1β 2 and nitric oxide enhances its paracrine effects on radiation-induced intestinal injury. Sci Rep 2015;5. doi: https://doi.org/10.1038/srep08718.
[138] Herrmann JL, Wang Y, Abarbanell AM, Weil BR, Tan J, Meldrum DR. Preconditioning mesenchymal stem cells with transforming growth factor-alpha improves mesenchymal stem cell-mediated cardioprotection. Shock 2010;33. doi: https://doi.org/10.1097/SHK.0b013e3181b7d137.
[139] Heo SC, Jeon ES, Lee IH, Kim HS, Kim MB, Kim JH. Tumor necrosis factor-α-activated human adipose tissue-derived mesenchymal stem cells accelerate cutaneous wound healing through paracrine mechanisms. J Invest Dermatol 2011;131:1559–67. doi: https://doi.org/10.1038/jid.2011.64.
[140] Li W, Liu Q, Shi J, Xu X, Xu J. The role of TNF-α in the fate regulation and functional reprogramming of mesenchymal stem cells in an inflammatory microenvironment. Front Immunol 2023;14. doi: https://doi.org/10.3389/fimmu.2023.1074863.
[141] Hackel A. Immunological priming of mesenchymal stromal/stem cells and their extracellular vesicles augments their therapeutic benefits in experimental graft-versus-host disease via engagement of PD-1 ligands. Front Immunol 2023;14. doi: https://doi.org/10.3389/fimmu.2023.1078551.
[142] Yu SP, Wei Z, Wei L. Preconditioning strategy in stem cell transplantation therapy. Transl Stroke Res 2013;4. doi: https://doi.org/10.1007/s12975-012-0251-0.
[143] Wang S, Umrath F, Cen W, Salgado AJ, Reinert S, Alexander D. Pre-conditioning with IFN-γ and hypoxia enhances the angiogenic potential of iPSC-derived MSC secretome. Cells 2022;11. doi: https://doi.org/10.3390/cells11060988.
[144] Lee SC, Jeong HJ, Lee SK, Kim SJ. Lipopolysaccharide preconditioning of adipose-derived stem cells improves liver-regenerating activity of the secretome. Stem Cell Res Ther 2015;6:1–11. doi: https://doi.org/10.1186/s13287-015-0072-7.
[145] Redondo-Castro E, Cunningham C, Miller J, Martuscelli L, Aoulad-Ali S, Rothwell NJ, et al. Interleukin-1 primes human mesenchymal stem cells towards an anti-inflammatory and pro-trophic phenotype in vitro. Stem Cell Res Ther 2017;8. doi: 10.1186/S13287-017-0531-4.
[146] Carrero R, Cerrada I, Lledó E, Dopazo J, García-García F, Rubio MP, et al. IL1β induces mesenchymal stem cells migration and leucocyte chemotaxis through NF-κB. Stem Cell Rev 2012;8:905. doi: https://doi.org/10.1007/S12015-012-9364-9.
[147] Bhang SH, Lee S, Shin JY, Lee TJ, Jang HK, Kim BS. Efficacious and clinically relevant conditioned medium of human adipose-derived stem cells for therapeutic angiogenesis. Mol Ther 2014;22. doi: https://doi.org/10.1038/mt.2013.301.
[148] Fuentes P, Torres MJ, Arancibia R, Aulestia F, Vergara M, Carrión F, et al. Dynamic culture of mesenchymal stromal/stem cell spheroids and secretion of paracrine factors. Front Bioeng Biotechnol 2022;10. doi: https://doi.org/10.3389/fbioe.2022.916229.
[149] Zhang X, Hu MG, Pan K, Li CH, Liu R. 3D spheroid culture enhances the expression of antifibrotic factors in human adipose-derived MSCs and improves their therapeutic effects on hepatic fibrosis 2016;2016. doi: 10.1155/2016/4626073.
[150] Cho YJ, Song HS, Bhang S, Lee S, Kang BG, Lee JC, et al. Therapeutic effects of human adipose stem cell-conditioned medium on stroke. J Neurosci Res 2012;90. doi: https://doi.org/10.1002/jnr.23063.
[151] Hodgkinson CP, Gomez JA, Mirotsou M, Dzau VJ. Genetic engineering of mesenchymal stem cells and its application in human disease therapy. Hum Gene Ther 2010;21:1513–26. doi: https://doi.org/10.1089/hum.2010.165.
[152] Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal stem cells for regenerative medicine. Cells 2019;8:886. doi: https://doi.org/10.1155/2014/951512.
[153] Dong YZ, Yang L Bin, Yang L, Zhao HX, Zhang C, Wu DP. Transplantation of neurotrophin-3-transfected bone marrow mesenchymal stem cells for the repair of spinal cord injury. Neural Regen Res 2014;9. doi: 10.4103/1673-5374.139478.
[154] Lai L, Chen J, Wei X, Huang M, Hu X, Yang R, et al. Transplantation of MSCs overexpressing HGF into a rat model of liver fibrosis. Mol Imaging Biol 2016;18:43–51. doi: https://doi.org/10.1007/s11307-015-0869-x.
[155] Liu X, Shen W, Yang Y, Liu G. Therapeutic implications of mesenchymal stem cells transfected with hepatocyte growth factor transplanted in rat kidney with unilateral ureteral obstruction. J Pediatr Surg 2011;46:537–45. doi: https://doi.org/10.1016/j.jpedsurg.2010.09.040.
[156] Wang K, Li Y, Zhu T, Zhang Y, Li W, Lin W, et al. Overexpression of c-Met in bone marrow mesenchymal stem cells improves their effectiveness in homing and repair of acute liver failure. Stem Cell Res Ther 2017;8:1–10. doi: https://doi.org/10.1186/s13287-017-0614-2.
[157] Ma HC, Shi XL, Ren HZ, Yuan XW, Ding YT. Targeted migration of mesenchymal stem cells modified with CXCR4 to acute failing liver improves liver regeneration. World J Gastroenterol 2014;20:14884–94. doi: https://doi.org/10.3748/WJG.V20.I40.14884.
[158] Katifelis H, Filidou E, Psaraki A, Yakoub F, Roubelakis MG, Tarapatzi G, et al. Amniotic fluid-derived mesenchymal stem/stromal cell-derived secretome and exosomes improve inflammation in human intestinal subepithelial myofibroblasts. Biomedicines 2022;10. doi: https://doi.org/10.3390/biomedicines10102357.
[159] Zhang L, Ye C, Li P, Li C, Shu W, Zhao Y, et al. ADSCs stimulated by VEGF-C alleviate intestinal inflammation via dual mechanisms of enhancing lymphatic drainage by a VEGF-C/VEGFR-3-dependent mechanism and inhibiting the NF-κB pathway by the secretome. Stem Cell Res Ther 2022;13. doi: 10.1186/s13287-022-03132-3.
[160] Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367–8. doi: 10.1038/nm0405-367.
[161] Ohnishi S, Sumiyoshi H, Kitamura S, Nagaya N. Mesenchymal stem cells attenuate cardiac fibroblast proliferation and collagen synthesis through paracrine actions. FEBS Lett 2007;581. doi: https://doi.org/10.1016/j.febslet.2007.07.028.
[162] Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor ENE, et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res 2013;10. doi: https://doi.org/10.1016/j.scr.2013.01.002.
[163] Guo Y, Bao S, Guo W, Diao Z, Wang L, Han X, et al. Bone marrow mesenchymal stem cell–derived exosomes alleviate high phosphorus-induced vascular smooth muscle cells calcification by modifying microRNA profiles. Funct Integr Genomics 2019. doi: https://doi.org/10.1007/s10142-019-00669-0.
[164] Qu Q, Pang Y, Zhang C, Liu L, Bi Y. Exosomes derived from human umbilical cord mesenchymal stem cells inhibit vein graft intimal hyperplasia and accelerate reendothelialization by enhancing endothelial function. Stem Cell Res Ther 2020;11. doi: 10.1186/s13287-020-01639-1.
[165] Osugi M, Katagiri W, Yoshimi R, Inukai T, Hibi H, Ueda M. Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. Tissue Eng Part A 2012;18. doi: https://doi.org/10.1089/ten.tea.2011.0325.
[166] Katagiri W, Kawai T, Osugi M, Sugimura-Wakayama Y, Sakaguchi K, Kojima T, et al. Angiogenesis in newly regenerated bone by secretomes of human mesenchymal stem cells. Maxillofac Plast Reconstr Surg 2017;39. doi: 10.1186/s40902-017-0106-4.
[167] Ando Y, Matsubara K, Ishikawa J, Fujio M, Shohara R, Hibi H, et al. Stem cell-conditioned medium accelerates distraction osteogenesis through multiple regenerative mechanisms. Bone 2014;61. doi: https://doi.org/10.1016/j.bone.2013.12.029.
[168] Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C. Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. Am J Physiol Renal Physiol 2005;289. doi: https://doi.org/10.1152/ajprenal.00007.2005.
[169] van Koppen A, Joles JA, van Balkom BWM, Lim SK, de Kleijn D, Giles RH, et al. Human embryonic mesenchymal stem cell-derived conditioned medium rescues kidney function in rats with established chronic kidney disease. PLoS One 2012;7. doi: 10.1371/journal.pone.0038746.
[170] Yang CY, Chang PY, Chen JY, Wu BS, Yang AH, Lee OKS. Adipose-derived mesenchymal stem cells attenuate dialysis-induced peritoneal fibrosis by modulating macrophage polarization via interleukin-6. Stem Cell Res Ther 2021;12. doi: 10.1186/s13287-021-02270-4.
[171] Conese M, Piro D, Carbone A, Castellani S, Di Gioia S. Hematopoietic and mesenchymal stem cells for the treatment of chronic respiratory diseases: Role of plasticity and heterogeneity. Sci World J 2014;2014. doi: 10.1155/2014/859817.
[172] Monsel A, Zhu YG, Gudapati V, Lim H, Lee JW. Mesenchymal stem cell derived secretome and extracellular vesicles for acute lung injury and other inflammatory lung diseases. Expert Opin Biol Ther 2016;16:859–71. doi: https://doi.org/10.1517/14712598.2016.1170804.
[173] Dushianthan A, Grocott MPW, Postle AD, Cusack R. Acute respiratory distress syndrome and acute lung injury. Postgrad Med J 2011;87:612–22. doi: https://doi.org/10.1136/pgmj.2011.118398.
[174] Harrell CR, Fellabaum C, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Molecular mechanisms responsible for therapeutic potential of mesenchymal stem cell-derived secretome. Cells 2019;8:467. doi: 10.3390/CELLS8050467.
[175] Ortiz LA, DuTreil M, Fattman C, Pandey AC, Torres G, Go K, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci 2007;104:11002–7. doi: https://doi.org/10.1073/PNAS.0704421104.

[176] Nataliya B, Mikhail A, Vladimir P, Olga G, Maksim V, Ivan Z, et al. Mesenchymal stromal cells facilitate resolution of pulmonary fibrosis by miR-29c and miR-129 intercellular transfer. Exp Mol Med 2023. doi: https://doi.org/10.1038/s12276-023-01017-w.
[177] Bari E, Ferrarotti I, Saracino L, Perteghella S, Torre ML, Richeldi L, et al. Mesenchymal stromal cell secretome for post-covid-19 pulmonary fibrosis: a new therapy to treat the long-term lung sequelae? Cells 2021;10. doi: https://doi.org/10.3390/cells10051203.
[178] Hu W, Yang J, Xue J, Ma J, Wu S, Wang J, et al. Secretome of hESC-derived MSC-like immune and matrix regulatory cells mitigate pulmonary fibrosis through antioxidant and anti-inflammatory effects. Biomedicines 2023;11. doi: https://doi.org/10.3390/biomedicines11020463.
[179] Castranova V, Rabovsky J, Tucker JH, Miles PR. The alveolar type II epithelial cell: a multifunctional pneumocyte. Toxicol Appl Pharmacol 1988;93:472–83. doi: https://doi.org/10.1016/0041-008X(88)90051-8.
[180] Monsel A, Zhu YG, Gennai S, Hao Q, Hu S, Rouby JJ, et al. Therapeutic effects of human mesenchymal stem cell-derived microvesicles in severe pneumonia in mice. Am J Respir Crit Care Med 2015;192:324–36. doi: https://doi.org/10.1164/rccm.201410-1765OC.
[181] Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, et al. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. Circulation 2012;126:2601–11. doi: https://doi.org/10.1161/CIRCULATIONAHA.112.114173.
[182] Romero-Gomez M. NAFLD and NASH: biomarkers in detection, diagnosis and monitoring. Springer International Publishing; 2020. doi: 10.1007/978-3-030-37173-9/COVER.
[183] Global Observatory on Donation and Transplantation (GODT) World Health Organization. Global Observatory on Donation and Transplantation (GODT) World Health Organization. Organ donation and transplantation activities 2016; 2016. http://www.transplant-observatory.org/ (accessed February 2, 2024).
[184] Varaa N, Azandeh S, Khodabandeh Z, Gharravi AM. Wharton's jelly mesenchymal stem cell: various protocols for isolation and differentiation of hepatocyte-like cells; narrative review. Iran J Med Sci 2019;44:437. doi: https://doi.org/10.30476/IJMS.2019.44952.
[185] Du Z, Wei C, Cheng K, Han B, Yan J, Zhang M, et al. Mesenchymal stem cell–conditioned medium reduces liver injury and enhances regeneration in reduced-size rat liver transplantation. J Surg Res 2013;183:907–15. doi: https://doi.org/10.1016/J.JSS.2013.02.009.
[186] Chen YX, Zeng ZC, Sun J, Zeng HY, Yan-Huang ZZY. Mesenchymal stem cell-conditioned medium prevents radiation-induced liver injury by inhibiting inflammation and protecting sinusoidal endothelial cells. J Radiat Res 2015;56:700–8. doi: https://doi.org/10.1093/JRR/RRV026.
[187] English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-γ and TNF-α differentially regulate immunomodulation by murine mesenchymal stem cells. Immunol Lett 2007;110:91–100. doi: https://doi.org/10.1016/J.IMLET.2007.04.001.
[188] Ryan JM, Barry F, Murphy JM, Mahon BP. Interferon-γ does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol 2007;149:353–63. doi: https://doi.org/10.1111/J.1365-2249.2007.03422.X.
[189] Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, et al. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci 2005;12:47–57. doi: https://doi.org/10.1007/S11373-004-8183-7.
[190] Di NM, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002;99:3838–43. doi: https://doi.org/10.1182/BLOOD.V99.10.3838.
[191] Parekkadan B, van Poll D, Megeed Z, Kobayashi N, Tilles AW, Berthiaume F, et al. Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells. Biochem Biophys Res Commun 2007;363:247–52. doi: https://doi.org/10.1016/J.BBRC.2007.05.150.
[192] Wang J, Bian C, Liao L, Zhu Y, Li J, Zeng L, et al. Inhibition of hepatic stellate cells proliferation by mesenchymal stem cells and the possible mechanisms. Hepatol Res 2009;39:1219–28. doi: https://doi.org/10.1111/J.1872-034X.2009.00564.X.
[193] Kang SH, Kim MY, Eom YW, Baik SK. Mesenchymal stem cells for the treatment of liver disease: present and perspectives. Gut Liver 2020;14:306. doi: https://doi.org/10.5009/GNL18412.
[194] Franceschini V, Bettini S, Pifferi S, Rosellini A, Menini A, Saccardi R, et al. Human cord blood CD133+ stem cells transplanted to nod-scid mice provide conditions for regeneration of olfactory neuroepithelium after permanent damage induced by dichlobenil. Stem Cells 2009;27:825–35. doi: https://doi.org/10.1002/STEM.11.
[195] Zagoura D, Trohatou O, Makridakis M, Kollia A, Kokla N, Mokou M, et al. Functional secretome analysis reveals Annexin-A1 as important paracrine factor derived from fetal mesenchymal stem cells in hepatic regeneration. EBioMedicine 2019;45:542–52. doi: https://doi.org/10.1016/J.EBIOM.2019.07.009.
[196] Tang BL. Sirt1 and the mitochondria. Mol Cells 2016;39:87–95. doi: https://doi.org/10.14348/MOLCELLS.2016.2318.
[197] Yang M, Cui Y, Song J, Cui C, Wang L, Liang K, et al. Mesenchymal stem cell-conditioned medium improved mitochondrial function and alleviated inflammation and apoptosis in non-alcoholic fatty liver disease by regulating SIRT1. Biochem Biophys Res Commun 2021;546:74–82. doi: https://doi.org/10.1016/J.BBRC.2021.01.098.
[198] Roehlen N, Crouchet E, Baumert TF. Liver fibrosis: mechanistic concepts and therapeutic perspectives. Cells 2020;9. doi: https://doi.org/10.3390/cells9040875.
[199] Kim YH, Cho KA, Park M, Kim HS, Park JW, Woo SY, et al. Conditioned medium from tonsil-derived mesenchymal stem cells relieves CCl 4-induced liver fibrosis in mice. Tissue Eng Regen Med 2018;16:51–8. doi: https://doi.org/10.1007/S13770-018-0160-8.
[200] Meier RPH, Mahou R, Morel P, Meyer J, Montanari E, Muller YD, et al. Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice. J Hepatol 2015;62:634–41. doi: https://doi.org/10.1016/J.JHEP.2014.10.030.
[201] Zhang LT, Fang XQ, Chen QF, Chen H, Xiao P, Bin PX, et al. Bone marrow-derived mesenchymal stem cells inhibit the proliferation of hepatic stellate cells by inhibiting the transforming growth factor β pathway. Mol Med Rep 2015;12:7227–32. doi: https://doi.org/10.3892/MMR.2015.4362.
[202] Rabani V, Shahsavani M, Gharavi M, Piryaei A, Azhdari Z, Baharvand H. Mesenchymal stem cell infusion therapy in a carbon tetrachloride-induced liver fibrosis model affects matrix metalloproteinase expression. Cell Biol Int 2010;34:601–5. doi: https://doi.org/10.1042/CBI20090386.
[203] An SY, Jang YJ, Lim HJ, Han J, Lee J, Lee G, et al. Milk fat globule-EGF factor 8, secreted by mesenchymal stem cells, protects against liver fibrosis in mice. Gastroenterology 2017;152:1174–86. doi: https://doi.org/10.1053/J.GASTRO.2016.12.003.
[204] Van Poll D, Parekkadan B, Cho CH, Berthiaume F, Nahmias Y, Tilles AW, et al. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. Hepatology 2008;47:1634–43. doi: https://doi.org/10.1002/HEP.22236.
[205] Xagorari A, Siotou E, Yiangou M, Tsolaki E, Bougiouklis D, Sakkas L, et al. Protective effect of mesenchymal stem cell-conditioned medium on hepatic cell apoptosis after acute liver injury. Int J Clin Exp Pathol 2013;6:831.
[206] Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci 2017;18:. doi: https://doi.org/10.3390/ijms18091852 1852.
[207] Gilgenkrantz H, Collin de l'Hortet A. New insights into liver regeneration. Clin Res Hepatol Gastroenterol 2011;35:623–9. doi: https://doi.org/10.1016/J.CLINRE.2011.04.002.
[208] Zheng J, Chen L, Lu T, Zhang Y, Sui X, Li Y, et al. MSCs ameliorate hepatocellular apoptosis mediated by PINK1-dependent mitophagy in liver ischemia/reperfusion injury through AMPKα activation. Cell Death Dis 2020;11:1–19. doi: 10.1038/s41419-020-2424-1.
[209] Oskowitz A, McFerrin H, Gutschow M, Carter ML, Pochampally R. Serum-deprived human multipotent mesenchymal stromal cells (MSCs) are highly angiogenic. Stem Cell Res 2011;6:215–25. doi: https://doi.org/10.1016/J.SCR.2011.01.004.
[210] Fouraschen SMG, Pan Q, De Ruiter PE, Farid WRR, Kazemier G, Kwekkeboom J, et al. Secreted factors of human liver-derived mesenchymal stem cells promote liver regeneration early after partial hepatectomy. Stem Cells Dev 2012;21:2410–9. doi: https://doi.org/10.1089/SCD.2011.0560.
[211] Choi JS, Ryu HA, Cheon SH, Kim SW. Human adipose derived stem cells exhibit enhanced liver regeneration in acute liver injury by controlled releasing hepatocyte growth factor. Cell Physiol Biochem 2019;52:935–50. doi: https://doi.org/10.33594/000000065.
[212] Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. Stem Cells Dev 2013;22:845–54. doi: https://doi.org/10.1089/scd.2012.0395.
[213] Tan CY, Lai RC, Wong W, Dan YY, Lim S-K, Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 2014;5:76. doi: https://doi.org/10.1186/scrt465.
[214] Boese AC, Le QSE, Pham D, Hamblin MH, Lee JP. Neural stem cell therapy for subacute and chronic ischemic stroke. Stem Cell Res Ther 2018;9:154. doi: https://doi.org/10.1186/s13287-018-0913-2.
[215] Kazanis I, Lathia J, Moss L, Ffrench-Constant C. The neural stem cell microenvironment. Stembook, Cambridge (MA): Harvard Stem Cell Institute; 2008. p. 1–26.
[216] Willis CM, Nicaise AM, Peruzzotti-Jametti L, Pluchino S. The neural stem cell secretome and its role in brain repair. Brain Res 2020;1729:. doi: https://doi.org/10.1016/j.brainres.2019.146615146615.
[217] Islam O, Gong X, Rose-John S, Heese K. Interleukin-6 and neural stem cells: more than gliogenesis. Mol Biol Cell 2009;20:188–99. doi: https://doi.org/10.1091/mbc.E08-05-0463.
[218] Severino V, Farina A, Colucci-D'Amato L, Reccia MG, Volpicelli F, Parente A, et al. Secretome profiling of differentiated neural mes-c-myc A1 cell line endowed with stem cell properties. Biochim Biophys Acta Proteins Proteom 2013;1834:2385–95. doi: https://doi.org/10.1016/j.bbapap.2012.12.005.
[219] De Gioia R, Biella F, Citterio G, Rizzo F, Abati E, Nizzardo M, et al. Neural stem cell transplantation for neurodegenerative diseases. Int J Mol Sci 2020;21:. doi: https://doi.org/10.3390/ijms21093103 3103.
[220] Lazarov O, Mattson MP, Peterson DA, Pimplikar SW, van Praag H. When neurogenesis encounters aging and disease. Trends Neurosci 2010;33:569. doi: https://doi.org/10.1016/J.TINS.2010.09.003.
[221] Badyra B, Sułkowski M, Milczarek O, Majka M. Mesenchymal stem cells as a multimodal treatment for nervous system diseases. Stem Cells Transl Med 2020;9:1174–89. doi: https://doi.org/10.1002/sctm.19-0430.

[222] Salgado AJ, Sousa JC, Costa BM, Pires AO, Mateus-Pinheiro A, Teixeira FG, et al. Mesenchymal stem cells secretome as a modulator of the neurogenic niche: basic insights and therapeutic opportunities. Front Cell Neurosci 2015;9:. doi: https://doi.org/10.3389/fncel.2015.00249249.
[223] Nowakowski A, Walczak P, Janowski M, Lukomska B. Genetic engineering of mesenchymal stem cells for regenerative medicine. Stem Cells Dev 2015;24:2219–42. doi: https://doi.org/10.1089/scd.2015.0062.
[224] Cofano F, Boido M, Monticelli M, Zenga F, Ducati A, Vercelli A, et al. Mesenchymal stem cells for spinal cord injury: current options, limitations, and future of cell therapy. Int J Mol Sci 2019;20:. doi: https://doi.org/10.3390/IJMS20112698 2698.
[225] Barati S, Tahmasebi F, Faghihi F. Effects of mesenchymal stem cells transplantation on multiple sclerosis patients. Neuropeptides 2020;84:. doi: https://doi.org/10.1016/J.NPEP.2020.102095102095.
[226] Gu Y, He M, Zhou X, Liu J, Hou N, Bin T, et al. Endogenous IL-6 of mesenchymal stem cell improves behavioral outcome of hypoxic-ischemic brain damage neonatal rats by supressing apoptosis in astrocyte. Sci Rep 2016:6. doi: https://doi.org/10.1038/srep18587.
[227] Walker CL. Adipose-derived stem cell conditioned medium for the treatment of amyotrophic lateral sclerosis: pre-clinical evidence and potential for clinical application. Neural Regen Res 2019;14:1522. doi: https://doi.org/10.4103/1673-5374.253514.
[228] Scalabrino G. Epidermal growth factor in the CNS: a beguiling journey from integrated cell biology to multiple sclerosis. An extensive translational overview. Cell Mol Neurobiol 2022;42:891–916. doi: https://doi.org/10.1007/s10571-020-00989-x.
[229] Kassis I, Grigoriadis N, Gowda-Kurkalli B, Mizrachi-Kol R, Ben-Hur T, Slavin S, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. Arch Neurol 2008;65:753–61. doi: https://doi.org/10.1001/ARCHNEUR.65.6.753.
[230] Wilkins A, Kemp K, Ginty M, Hares K, Mallam E, Scolding N. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro. Stem Cell Res 2009;3:63–70. doi: https://doi.org/10.1016/J.SCR.2009.02.006.
[231] Pisati F, Bossolasco P, Meregalli M, Cova L, Belicchi M, Gavina M, et al. Induction of neurotrophin expression via human adult mesenchymal stem cells: implication for cell therapy in neurodegenerative diseases. Cell Transplant 2007;16:41–55. doi: https://doi.org/10.3727/000000007783464443.
[232] Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. Brain Res Rev 2008;59:201–20. doi: https://doi.org/10.1016/j.brainresrev.2008.07.007.
[233] Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 2001;24:677–736. doi: https://doi.org/10.1146/annurev.neuro.24.1.677.
[234] Minnone G, De Benedetti F, Bracci-Laudiero L. NGF and its receptors in the regulation of inflammatory response. Int J Mol Sci 2017:18. doi: https://doi.org/10.3390/IJMS18051028.
[235] Kato T. Biological roles of hepatocyte growth factor-Met signaling from genetically modified animals. Biomed Rep 2017;7:495–503. doi: https://doi.org/10.3892/BR.2017.1001.
[236] Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranso J, et al. Hepatocyte growth factor mediates MSCs stimulated functional recovery in animal models of MS. Nat Neurosci 2012;15:862. doi: https://doi.org/10.1038/NN.3109.
[237] Mendes-Pinheiro B, Anjo SI, Manadas B, Da Silva JD, Marote A, Behie LA, et al. Bone marrow mesenchymal stem cells' secretome exerts neuroprotective effects in a Parkinson's disease rat model. Front Bioeng Biotechnol 2019;1:. doi: https://doi.org/10.3389/FBIOE.2019.00294294.
[238] Chen HX, Liang FC, Gu P, Xu BL, Xu HJ, Wang WT, et al. Exosomes derived from mesenchymal stem cells repair a Parkinson's disease model by inducing autophagy. Cell Death Dis 2020;11:1–17. doi: 10.1038/s41419-020-2473-5.
[239] Niu Y, Xia X, Song P, Fang H, Dong F, Tao H, et al. Bone mesenchymal stem cell-conditioned medium attenuates the effect of oxidative stress injury on NSCs by inhibiting the Notch1 signaling pathway. Cell Biol Int 2019;43:1267–75. doi: https://doi.org/10.1002/CBIN.11126.
[240] Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. J Hematol Oncol 2021;14 (1):1–24. doi: https://doi.org/10.1186/S13045-021-01037-X.
[241] Rezabakhsh A, Sokullu E, Rahbarghazi R. Applications, challenges and prospects of mesenchymal stem cell exosomes in regenerative medicine. Stem Cell Res Ther 2021:12. doi: https://doi.org/10.1186/s13287-021-02596-z.
[242] Giovannelli L, Bari E, Jommi C, Tartara F, Armocida D, Garbossa D, et al. Mesenchymal stem cell secretome and extracellular vesicles for neurodegenerative diseases: risk-benefit profile and next steps for the market access. Bioact Mater 2023:29. doi: https://doi.org/10.1016/j.bioactmat.2023.06.013.
[243] Teixeira F, Salgado A. Mesenchymal stem cells secretome: current trends and future challenges. Neural Regen Res 2020;15:75–7. doi: https://doi.org/10.4103/1673-5374.264455.
[244] Chouaib B, Haack-Sørensen M, Chaubron F, Cuisinier F, Collart-Dutilleul PY. Towards the standardization of mesenchymal stem cell secretome-derived product manufacturing for tissue regeneration. Int J Mol Sci 2023;24:12594. doi: 10.3390/IJMS241612594.
[245] Guess AJ, Daneault B, Wang R, Bradbury H, La Perle KMD, Fitch J, et al. Safety profile of good manufacturing practice manufactured interferon γ-primed mesenchymal stem/stromal cells for clinical trials. Stem Cells Transl Med 2017;6:1868–79. doi: https://doi.org/10.1002/SCTM.16-0485.
[246] Carvalho MS, Alves L, Bogalho I, Cabral JMS, da Silva CL. Impact of donor age on the osteogenic supportive capacity of mesenchymal stromal cell-derived extracellular matrix. Front Cell Dev Biol 2021;9:. doi: https://doi.org/10.3389/FCELL.2021.747521/BIBTEX747521.
[247] LeBlon CE, Casey ME, Fodor CR, Zhang T, Zhang X, Jedlicka SS. Correlation between in vitro expansion-related cell stiffening and differentiation potential of human mesenchymal stem cells. Differentiation 2015;90:1–15. doi: https://doi.org/10.1016/J.DIFF.2015.08.002.
[248] Zaim M, Karaman S, Cetin G, Isik S. Donor age and long-term culture affect differentiation and proliferation of human bone marrow mesenchymal stem cells. Ann Hematol 2012;91:1175–86. doi: https://doi.org/10.1007/S00277-012-1438-X.
[249] Kolliopoulos V, Tiffany A, Polanek M, Harley BAC. Donor variability in human mesenchymal stem cell osteogenic response as a function of passage conditions and donor sex. BioRxiv 2023. doi: https://doi.org/10.1101/2023.11.12.566781.
[250] Arifka M, Wilar G, Elamin KM, Wathoni N. Polymeric hydrogels as mesenchymal stem cell secretome delivery system in biomedical applications. Polymers (Basel) 2022:14. doi: https://doi.org/10.3390/POLYM14061218.
[251] Racchetti G, Meldolesi J. Extracellular vesicles of mesenchymal stem cells: therapeutic properties discovered with extraordinary successok. Biomedicines 2021:9. doi: https://doi.org/10.3390/biomedicines9060667.
[252] Saleh AF, Lázaro-Ibáñez E, Forsgard MAM, Shatnyeva O, Osteikoetxea X, Karlsson F, et al. Extracellular vesicles induce minimal hepatotoxicity and immunogenicity. Nanoscale 2019;11:6990–7001. doi: https://doi.org/10.1039/C8NR08720B.
[253] Banks WA, Sharma P, Bullock KM, Hansen KM, Ludwig N, Whiteside TL. Transport of extracellular vesicles across the blood-brain barrier: brain pharmacokinetics and effects of inflammation. Int J Mol Sci 2020;21:4407. doi: 10.3390/IJMS21124407.
[254] de Godoy MA, Saraiva LM, de Carvalho LRP, Vasconcelos-dos-Santos A, Beiral HJV, Ramos AB, et al. Mesenchymal stem cells and cell-derived extracellular vesicles protect hippocampal neurons from oxidative stress and synapse damage induced by amyloid-β oligomers. J Biol Chem 2018;293:1957–75. doi: https://doi.org/10.1074/JBC.M117.807180.
[255] Wang JH, Liu XL, Sun JM, Yang JH, Xu DH, Yan SS. Role of mesenchymal stem cell derived extracellular vesicles in autoimmunity: a systematic review. World J Stem Cells 2020;12:. doi: https://doi.org/10.4252/WJSC.V12.I8.879879.
[256] Wang C, Börger V, Sardari M, Murke F, Skuljec J, Pul R, et al. Mesenchymal stromal cell-derived small extracellular vesicles induce ischemic neuroprotection by modulating leukocytes and specifically neutrophils. Stroke 2020;51:1825–34. doi: https://doi.org/10.1161/STROKEAHA.119.028012.
[257] Silachev DN, Goryunov KV, Shpilyuk MA, Beznoschenko OS, Morozova NY, Kraevaya EE, et al. Effect of MSCs and MSC-derived extracellular vesicles on human blood coagulation. Cells 2019:. doi: https://doi.org/10.3390/cells80302588.
[258] Mehryab F, Taghizadeh F, Goshtasbi N, Merati F, Rabbani S, Haeri A. Exosomes as cutting-edge therapeutics in various biomedical applications: an update on engineering, delivery, and preclinical studies. Biochimie 2023:213. doi: https://doi.org/10.1016/j.biochi.2023.05.010.
[259] Parada N, Romero-Trujillo A, Georges N, Alcayaga-Miranda F. Camouflage strategies for therapeutic exosomes evasion from phagocytosis. J Adv Res 2021;31:61–74. doi: https://doi.org/10.1016/J.JARE.2021.01.001.
[260] Desole C, Gallo S, Vitacolonna A, Montarolo F, Bertolotto A, Vivien D, et al. HGF and MET: From Brain Development to Neurological Disorders. Front Cell Dev Biol 2021;9. doi: https://doi.org/10.3389/fcell.2021.683609.
[261] Göbel K, Ruck T. Meuth SG Cytokine signaling in multiple sclerosis: Lost in translation. Mult Scler 2018;24:432–9. doi: https://doi.org/10.1177/1352458518763094.
[262] Keane C, Jerkic M, Laffey JG. Stem Cell–based Therapies for Sepsis. Anesthesiology 2017;127:1017–34. doi: https://doi.org/10.1097/ALN.0000000000001882.
[263] Ratajczak P, Janin A, Peffault de Larour R, Koch L, Roche B, Munn D, et al. IDO in Human Gut Graft-versus-Host Disease. Biology of Blood and Marrow Transplant 2012;18:150–5.
[264] Jalili R, Forouzandeh F, Bahar M, Ghahary A. The Immunoregulatory Function of Indoleamine 2, 3 Dioxygenase and Its Application in Allotransplantation. Iran J Allergy Asthma Immunol 2007;6:167–79.
[265] Varkouhi AK, Jerkic M, Ormesher L, Gagnon S, Goyal S, Rabani R, et al. Extracellular Vesicles from Interferon-γ–primed Human Umbilical Cord Mesenchymal Stromal Cells Reduce Escherichia coli–induced Acute Lung Injury in Rats. Anesthesiology 2019;130:778–90. doi: https://doi.org/10.1097/ALN.0000000000002655.
[266] Yan Y, Jiang W, Tan Y, Zou S, Zhang H, Mao F, et al. hucMSC Exosome-Derived GPX1 Is Required for the Recovery of Hepatic Oxidant Injury. Molecular Therapy 2017;25:465–79.
[267] Jiang L, Zhang S, Hu H, Yang J, Wang XY, Ma Y, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate acute liver failure by

reducing the activity of the NLRP3 inflammasome in macrophages. Biochem Biophys Res Commun 2019;508:735–41. doi: https://doi.org/10.1016/j.bbrc.2018.11.189.
[268] Liu Y, Lou G, Li A, Zhang T, Qi J, Ye D, et al. AMSC-derived exosomes alleviate lipopolysaccharide/d-galactosamine-induced acute liver failure by miR-17-mediated reduction of TXNIP/NLRP3 inflammasome activation in macrophages. EBioMedicine 2018;36:140–50.
[269] Rong X, Liu J, Yao X, Jiang T, Wang Y, Xie F. Human bone marrow mesenchymal stem cells-derived exosomes alleviate liver fibrosis through the Wnt/β-catenin pathway. Stem Cell Res Ther 2019;10. doi: https://doi.org/10.1186/s13287-019-1204-2.
[270] Mardpour S, Hassani SN, Mardpour S, Sayahpour F, Vosough M, Ai J, et al. Extracellular vesicles derived from human embryonic stem cell-MSCs ameliorate cirrhosis in thioacetamide-induced chronic liver injury. J Cell Physiol 2018;233:9330–9344.doi. doi: https://doi.org/10.1002/jcp.26413.
[271] Shiue SJ, Rau RH, Shiue HS, Hung YW, Li ZX, Yang KD, et al. Mesenchymal stem cell exosomes as a cell-free therapy for nerve injury-induced pain in rats. Pain 2019;160:210–23. doi: https://doi.org/10.1097/j.pain.0000000000001395.

Catarina M. Trigo has a MSc in Pharmaceutical Sciences from Faculty of Pharmacy, University of Lisbon (FFUL), since 2020. Currently, she is a PhD student developing her project at Research Institute for Medicines (imed. ULisboa), FFUL, under the supervision of Prof. Joana Miranda and co-supervision of Prof. Susana Solá and Prof. João Gonçalves. Her PhD work aims at exploring the regenerative potential of mesenchymal stem cell-derived secretome to modulate neuroinflammatory pathologies, including to reduce the neurological sequelae associated with COVID-19. Since the beginning of her PhD, Catarina has five conference abstracts, one oral communication and two posters. She is member of the Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC) and of Pharmacology (SPF), and of Portuguese Network on Extracellular Vesicles (PNEV).

Joana S. Rodrigues holds a Biomedical Engineering degree from Instituto Superior Técnico. She is a PhD student at Faculty of Pharmacy, University of Lisbon (FFUL), under the supervision of Prof. Joana Miranda and Prof. Jorge Ruas, since 2020. She is developing his research at Advanced Cell Models for Predictive Toxicology & Cell-based Therapies Laboratory, Research Institute for Medicines (imed.ULisboa), FFUL and Karolinska Institutet. Her main research interest is the development of hepatic *in vitro* models for predictive toxicology and disease modelling using human stem cell-derived hepatocyte-like cells (HLCs). She is member of the Portuguese Society of Pharmacology (SPF) and the Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC) and author of 6 peer-reviewed manuscripts in international journals, 1 book chapter, 1 patent, and 12 published abstracts. Her h-index is 4 from Scopus.

Sérgio P. Camões received his PhD in Pharmacy from Faculty of Pharmacy, University of Lisbon (FFUL), in 2020. He is currently a post-doc researcher at Advanced Cell Models for Predictive Toxicology & Cell-based Therapies Laboratory, Research Institute for Medicines (imed.ULisboa), FFUL. His scientific career has been focused on the development of innovative therapeutic approaches in the field of regenerative medicine. He has been interested in how mesenchymal stem cells and their secretome, including extracellular vesicles, can be modulated towards a healing-prone phenotype by using 3D culturing and loading techniques for cell and cell-free therapy purposes. He is member of Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC), of Pharmacology (SPF), and of Immunology (SPI), and International Society for Extracellular Vesicles (ISEV). To date, he authored 14 original peer-reviewed manuscripts in international journals, 2 book chapters, 2 patents (1 international), and 18 published abstracts. His h-index is 7 from Scopus.

Susana Solá received her PhD in Pharmacy from the Faculty of Pharmacy, University of Lisbon (FFUL), after PhD training at the University of Minnesota. Currently, she is Associate Professor at FFUL, and leader of the Stem Cell Bioenergetics and Neuroregeneration Group at Research institute for Medicines (imed.ULisboa), FFUL. She is also member of Scientific Societies and European Consortia, e.g., board member of the Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC), the International Society for Stem Cell Research and COST Actions. She has been dedicated in *i)* understanding the molecular mechanisms involved in neural stem cell (NSC) fate, *ii)* identifying potential checkpoints and promising molecules that increase the activity of these cells during neuroregeneration, and *iii)* exploring the relevance of metabolism in inter-organ communication to improve the activity of NSCs and ameliorate neuroplasticity in adulthood. Her work granted her several important distinctions, namely Portuguese National UNESCO Commission (CNU). To date, she has authored more than 60 publications and has an h-index of 32 from Scopus.

Joana P. Miranda holds a PhD in Biochemistry and Biotechnology from ITQB, Universidade Nova de Lisboa (2006). She is an Associate Professor at Faculty of Pharmacy, University of Lisbon (FFUL) and since 2021, she is head of Advanced cell models for predictive toxicology & cell-based therapies laboratory at Research institute for Medicines (imed.ULisboa), FFUL. She is also a board member COST actions, of the Portuguese Society of Stem Cells and Cell Therapy (SPCE-TC), is the National Co-coordinator of the European Registered Toxicologist since 2017, is the Chair of the Communication SubComittee of the EUROTOX and has served as coordinator of the toxicology section of the Portuguese Society of Pharmacology (SPF) until 2019. Her main research interests are 3D models, stem cell biology for predictive toxicology and regenerative medicine. Specifically, her research has been focusing in *i)* understanding the molecular mechanisms involved in mesenchymal stem cells regenerative potential, *ii)* identifying the key players in MSC/cellular crosstalk, and *iii)* exploring the relevance of 3D systems for improving cells metabolism and function.
Since 2010, Joana Miranda has participated in 13 funded projects (6 as PI). She also authored more than 50 peer-reviewed articles, 4 patents, 5 book chapters, 49 published abstracts, 11 invited lectures and has an h-index of 22 from the Scopus.