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Mesenchymal Stem Cells on Horizon: A New Arsenal of Therapeutic Agents

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Abstract
Over 10 years, mesenchymal stem cells (MSCs) have been considered as valuable and suitable cells for cell-based therapy applications, particularly in clinical trials. In any case, they are as yet not utilized routinely in clinics. At first, it was believed that MSCs play their roles, especially in regenerative medicine due to their differentiation and cell replacement properties. Interestingly, it is well-known that MSCs mainly exert their therapeutic effects through their vast bioactive factors. These findings turned scientists' consideration toward cell-free therapy concepts. From this point of view, MSCs can be considered as an arsenal of natural bioreactors in variety of therapeutic agents. MSCs inherently express various important therapeutic agents such as growth factors and cytokines that can be manufactured, handled and stored as a prepared-to-go biologic product. In this review, we provide a vision, highlight as well as discuss in order to introduce competitive natural robust bioreactor MSCs on the horizon.

Keywords MSC · Secretome · Cell-free therapy · Condition medium · Therapeutic agents

Introduction

Mesenchymal Stem Cells (MSCs) are multipotent stem cells that have a well-defined capacity for self-renewal [1]. MSCs express CD73, CD105, and CD90, while they have no expression of CD34, CD45, CD14 or CD11b, CD19a, and HLA-DR surface markers. Moreover, they have the capacity to differentiate into adipocytes, osteoblasts, and chondroblasts *in vitro* [2, 3]. Over the past decade, MSCs have gained much more attention in regenerative medicine area because of homing ability, immune regulatory properties [4], lower ethical concerns, and tumorigenicity [5], as well as, transdifferentiation capacity [6], tissue-organ repairing, and promoting the survival of damaged tissues [7]. In addition, MSCs-based therapies have been performed in a number of trials worldwide [8] (https://clinicaltrials.gov).

In spite of many advantages of MSCs for cell therapy purposes, there are several challenges for clinical use. Some of them are; uncontrolled cell quality, invasive cell isolation process, loss of potency, limited lifespan, the gradual loss of their initial properties during expansion and *in vitro* proliferation. It is noteworthy that, stressful conditions such as oxidative stress, serum deprivation, inflammation, chemotherapy and radiotherapy in the recipients' tissues/organs, decrease the cell's survival dramatically, resulting in the death of around 99% of MSCs during a few days after transplantation [9–18]. Inadequate quantity of transplanted cells is another remarkable limitation of MSCs application. Noteworthy, more than one hundred million of MSCs require for cell therapy and it takes about ten weeks to prepare sufficient cells before transplantation. Furthermore, clinical features and age of patients are other concerns which influence on the optimal culture conditions of MSCs [19, 20]. In other words, aging and senescence phenotype in MSCs are other important limitations for clinical use. It has been shown that MSCs from aged-patients exhibit characteristics of aging and senescence such as epigenetic modifications, DNA mutations, mitochondrial dysfunction, and telomere length [21]. It has also been revealed that growth kinetic of adipose-derived stromal/stem cells (ASCs) is positively correlated with the donor's age. The proliferation of MSCs decreased in elderly

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people whereas apoptosis increased. Moreover, it seems that the differentiation potentials of MSCs change with increased age [16, 22, 23]. These observations indicate a necessary efficient solution to rejuvenate MSCs in vitro when clinical applications of them are considered.

Moreover, some evidence indicating that MSCs have a cancerous origin in the body tissues [24, 25]. In other words, there are common characteristics between MSCs and cancer stem cells that may result in tumorigenesis, which make them unsuitable for direct utilize in the clinic.

Furthermore, the effect of metabolic disorders on MSC's fate could be other limitation of them in clinical application. In other words, metabolic disorders may cause an effect on quality of MSCs. It has been shown that MSCs harvested from equine with metabolic disorders had lower proliferation rate, mitochondrial dysfunction, and higher autophagy cell death in comparison with healthy equine. Overall, the results of this study indicated that autologous MSCs transplantation could be challengeable in patients who are suffering from metabolic disorder [26].

On the other hand, a body of studies indicates that the therapeutic properties of MSCs are owing to their paracrine effects of growth and nutritional factors. Other studies have also shown that the stem cell-derived secreted agents are able to exert therapeutic effects without presence of any other cells [27–29]. MSCs' secretory trophic factors, hormones, and cytokines are known as secretome that can be produced in environment which stem cells are grown; which named that, conditioned medium (CM) [4] (Fig. 1). Note that, exosomes are part of the MSC secretome.

Therefore, in recent years, MSCs-derived secretome has been introduced as a promising candidate for novel cell-free therapy. For example, Camussi et al. reported that MSC's secretome prohibited kidney injury [30, 31]. In addition, it has been shown that the MSC exosomes of mice exert therapeutic effects to improve pulmonary hypertension in lung tissue [32, 33]. Other studies also indicate the MSC's secretome therapy promoted re-epithelialization of cutaneous wounds by inducing epithelial cell proliferation [34] and angiogenesis [35]. Several studies have been shown the presence of cytokines, hormones and growth factors in MSCs-derived CM which resulted in repairing of damaged tissues [36–45].

Here, we are going to introduce MSCs as an arsenal of therapeutic, beneficial and high-performance agents. In other words, we discuss and highlight the presence of various growth factors/cytokines and tissue regenerative factors, that making the MSCs as a natural, valuable, promising and versatile bioreactor in order to produce pharmaceuticals agents.

Secretome as a Novel Approach for Cell-free Therapy

MSCs have the ability in order to produce a wide range of chemokines, cytokines, growth factors and extracellular

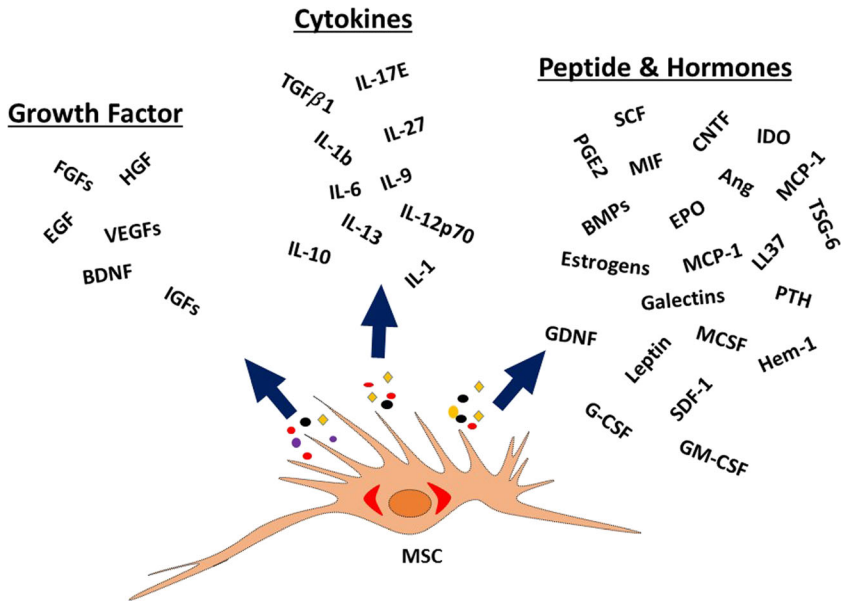

Fig. 1 MSCs secretory trophic factors, hormones, and cytokines are known as secretome. Growth factors: BDNF; Brain-Derived Neurotrophic Factor, EGF; Epidermal Growth Factor, FGFs; Fibroblast Growth Factor, HGF; Hepatocyte Growth Factor, IGFs; Insulin-Like Growth Factor, VEGF; Vascular Endothelial Derived Growth Factor. Cytokines: TGF-β1; Transforming Growth Factor Beta, IL-6; Interleukin 6, IL-10; Interleukin 10, IL-27; Interleukin 27, IL-17E; Interleukin 17E, IL-13; Interleukin 13, IL-1Ra; Interleukin 1 receptor antagonist, IL-8;Interleukin 8, IL-9; Interleukin 9, IL-1b; Interleukin-1b. Peptide & hormones: Ang; Angiopoietin, BMPs; Bone Morphogenetic Proteins, CNTF; Ciliary Neurotrophic Factor, EPO; Erythropoietin, GDNF; Glial cell line-Derived Neurotrophic Factor, G-CSF; Granulocyte Colony Stimulating Factor, GM-CSF; Granulocyte Macrophage CSF, MCSF; Macrophage CSF, HO-1; Hemoxygenase-1, IDO; Indoleamine 2,3-Dioxygenase, MCP-1; Monocyte chemotactic Protein, MIF; Macrophage Migration Inhibitory Factor, PGE*2*; Prostaglandin E2, SCF; Stem Cell Factor, SDF-1; Stromal Cell-Derived Factor 1, TSG-6; (TNF)-Stimulated Gene-6, LL37

Fig. 2 Summary of advantages of secretome. It can be manufactured, freeze-dried, packaged, and transported more easily. Moreover, as it is free of cells there is no need to match the donor and the recipient to avoid rejection problems

matrix (ECM) molecules. The MSC's niche including a variety of microenvironmental signals which are generated during healing, development or diseases, in turn, it regulates tissue regeneration through proliferation and differentiation [46]. The secretome is defined as a series of molecular factors which is secreted into extracellular space. These factors consist of hormones, soluble proteins, cytokines and growth factors [47]. The scientific evidence indicates that similar to the cellular counterpart, MSC's secretome can be used in order to exert favorable effects in tissue regeneration [48]. In other words, cytokines and growth factors produced in MSCs can be used for cell-free regenerative medicine. Interestingly, each cytokine and growth factor can be considered as a novel potential therapeutic agent [48]. Therefore, it may have a significant impact in the near future. Table 1 indicates the studies dealing with the usage of CM for treatment of a variety of diseases. However, depend on the tissues that MSC's CM/secretome have been isolated the contents of them are variable.

Advantages of Secretome as a Therapeutic Agent

As previously mentioned, the secretome is cell-free. Therefore, in allogeneic usage, it will reduce the risk of adverse reactions. On the other hand, secretome's therapeutic doses can be achieved with one million of MSCs. Secretome can be stored at −80 °C without any significant loss of quality and function which is ready to use after thawing immediately [50, 61]. In terms of stability, some growth factors/cytokines such as IL-6 are stable; IFN-γ and MIP-2 are somewhat stable at 4 °C and TNF-α, IL-10 and IL-17A are not stable in secretomes. Therefore, secretome containing IL- 10 and IL-17A can be stored at −80 °C; however, it is recommended to measure after the first thaw [62]. However, regarding with stability of secretome further studies are required.

The secretome-based therapy can be performed at the regular intervals for a long time, for example at weekly intervals, providing therapeutic courses instead of a single therapeutic at only one- time point. Figure 2 shows some advantages of secretome in regenerative medicine [50, 61, 62].

Secretome-based Therapy in Regenerative Medicine

A number of studies have been shown that secretome contains of immune-modulatory, anti-inflammatory, anti-apoptotic, anti-oxidant, anti-fibrotic, anti-bacterial and neuroprotective properties. Therefore, it can be employed in a variety of diseases. Moreover, it represents a ready-to-use therapeutic agent. Table 2 shows some current therapeutic applications in regenerative medicine. It is noteworthy that the majority of studies dealing with employing of secretome are in preclinical studies. Overall, these studies indicate that secretome, like. MSCs, is applicable for curing many of diseases.

MSCs as a Natural Arsenal of Therapeutic Agents

The various secretory agents produced by MSCs in the microenvironment could have a therapeutic potential. These secretory agents include growth factors, pro-inflammatory and anti-inflammatory cytokines, as well as other peptides and hormones. As mentioned before, it's

Table 1 Summary of studies in which MSCs-derived conditioned medium (MSCs-CM) are employed to treat in a variety of diseases

Diseases	Donor cells-derived CM	Clinical trials or preclinical	Rout of injection	Outcome	References
Spinal cord injury	BMMSCs	Preclinical/ Rat	Directly injection	- Increased angiogenesis - Protected neurons from apoptosis - Improved functional recovery after SCI *in vivo* - Reduced cystic cavity *in vivo*	[49]
Corneal epithelial wound healing	hUCESCs	Preclinical / Rat	Topically (1 drop)	- Anti-inflammatory effects -Bactericidal effects -Enhancing corneal wound healing	[36]
Uveitis	hUCESCs	Preclinical / Rat	Topically (1 drop)	-Reduced leucocytes -Reduced ocular inflammation	[50]
Acute and chronic hind limb ischemia	ADSCs	Preclinical / Mouse	Intramuscular	- Reduced apoptosis -Increased angiogenesis -Enhancement of endothelial cell growth -Improvement of blood perfusion in the hind limb ischemia	[37]
Cerebral injury	BMMSCs	Preclinical / Sprague–Dawley Rat	Intravenous	-Attenuated brain damaged volume - Attenuated incidence during TBI (traumatic brain injury) -Decreased TBI-induced neuronal apoptosis -Increased neurogenesis (under normoxic or hypoxic conditions)	[51]
Lung injury	BMMSCs	Preclinical / Mouse	Intratracheally	- Decreased LPS-induced lung injury with soluble factors IGF-I - Decreased lung inflammation - Decreased lung vascular permeability	[52]
Bone defects	BMMSCs	Clinical	Surgical/ Maxillary sinus floor elevation (SFE) (lateral window approach)	-Increase bone volume in the center of the augmented area -Clinically confirmation of bone formation in all cases	[53]
Colitis	Amniotic fluid	Preclinical / Mouse	Intraperitoneal	- Decreased the inflammation - Decreased levels of MMP2 protein -Increased levels of TGFb1	[54]
Alopecia	ADSCs	Preclinical/ Nude Mice	Subcutaneous	-Hair growth stimulation effects -Increased growth factor secretion in hypoxic CM	[39]
Muscular degenerative	ADSCs	Preclinical/ Rat	Local / systemic injection	-Decreased atrophy of the rotator cuff muscles -Prevented muscle degeneration.	[55]
Myocardial infarction	ADSCs	Preclinical/ Pig	Intravenous	-Improved Systolic and diastolic cardiac performance -Reduced myocardial oxidative stress -Reduced apoptosis	[56]
Acute liver injury/ failure	Amniotic fluid	Preclinical/ Nude Mice	Intrahepatically	-Induced liver recovery with presence of anti-inflammatory factors	[38]
Skin wound	ADSCs	Human clinical study /Original	Topically	- Reduced erythema and pigmentation -Fasten recovery of the skin barrier function -Enhancing wound healing	[57]
	PDLSCs	Preclinical/ Nude Mice	Intravenous		[58]

Table 1 (continued)

Diseases	Donor cells-derived CM	Clinical trials or preclinical	Rout of injection	Outcome	References
Multiple sclerosis				-Augmented neurons spine density and remyelination -Induced anti-inflammatory and immunosuppressive effects - Attenuated apoptosis -Immunosuppressive effects -Protected against EAE (experimental autoimmune encephalomyelitis)	
Parkinson's disease	WJMSCs	Preclinical/ Rat	Directly into the medial forebrain bundle (MFB) - Impact on brain structure and animal behavior - Restored the Neuronal Structure in PD		[59]
Atrophied muscles	UCPVCs	Preclinical/ Rat	Intramuscular	-Suppressed atrophy-related ubiquitin E3-ligases, MuRF-1, and MAFbx. - Regenerated muscle mass and proteins in atrophied muscles.	[60]
Cancer	hUCESCs	Preclinical/ Mouse	Intratumorally	-Modified cell cycle: increased G0-G1 phase, decreased G2-M, decreased expression of cyclin A, cyclin B, and cyclin D1 proteins -Induced apoptosis in the MDA-MB-231 cell line -Inhibited invasion, 3D growth, and tumor volume - Reduced proliferation in breast tumors	[61]

BMMSCs: Bone marrow-derived mesenchymal stem cells; ADSCs: Adipose-derived stem cells; DPSC: Dental pulp stem cells; WJMSCs: Umbilical cord Wharton's Jelly mesenchymal stem cells; hUCESCs: Human uterine cervical stem cells; UCPVCs: Umbilical cord perivascular cells; PDLSCs: Periodontal ligament stem cells

Table 2 Applications of secretome therapy

Disease	Tissue/Model	Outcome	Source of CM/Target	Reference
Circulatory system injury	Heart/Infarct, IR	-Cardio protective activity -Effective in animal models of myocardial infarction	-Human /Pig -Human /Mouse -Rat/Rat	[56, 63, 64]
Nervous system injury	Brain/Stroke, Ischemia	-Effective in stroke -Effective in peri-natal hypoxic-ischemic brain injury -Effective in hind-limb ischemia -Exhibited potent neuro protective activities in neurons - Models of traumatic brain injury	-Rat /Rat -Human /Ovine -Human /Mouse	[65–67]
Digestive system injury	Liver/fibrosis	-Ameliorated carbon tetrachloride -induced liver fibrosis -Conferred cyto-protective effects in models of necrotizing enterocolitis -Protected against experimental colitis	-Human /Mouse -Human /Rat -Rat /Rat	[68–70]
Respiratory system injury	-Lung / hypoxia, E.coli endotoxin, silicosis, fluid filled	-Effective in improving pulmonary hypertension -Improved endotoxin-induced pulmonary edema -Effective in improving silicosis -Cleared alveolar fluid from human lungs ex vivo	-Mouse /Mouse -Human /Mouse - Human /Human	[33, 71–73]
Skin and subcutaneous tissue injury	Skin /wound	-Promoted re-epithelialization of cutaneous wounds by inducing epithelial cell proliferation -Activated collagen and elastin secretion by fibroblasts -Prevented myo-fibroblast formation thereby reducing scaring	-Human /Rat -Human /Mouse	[34, 74, 75]
Musculoskeletal system and connective tissue injury	-Skeletal Muscle/cardiotoxin	Promoted muscle regeneration	-Human /Mouse	[76, 77]
Acute kidney injury (AKI)		-Attenuated renal injury -Improved kidney recovery competence -Attenuated necrosis, apoptosis, and inflammation -Increased cellular proliferation	-Human /Pig - Human /Mouse - Human /Rat	[78–80]

Table 3 Some trophic factors and cytokines produced by MSCs and suggested functions for tissue regeneration/repair

Abbreviation	Full name	Functions	Reference
Growth factor			
BDNF	Brain Derived Neurotrophic Factor	Promotes survival and differentiation of neurons, reduces infarct size	[49]
EGF	Epidermal Growth Factor	Induces cell proliferation and differentiation	[38, 39, 41, 42]
FGF	Fibroblast Growth Factor 2/Basic Fibroblast Growth Factor (FGF-2/BFGF)	Induces angiogenesis, inhibit apoptosis	[16, 37, 41, 42, 86]
HGF	Hepatocyte Growth Factor	Promotes progenitor cell mobilization, induces angiogenesis and cell proliferation, inhibits immune cell proliferation	[37, 38, 45, 87, 88]
IGF	Insulin-Like Growth Factor I (IGF-I) Insulin-Like Growth Factor II (IGF-II)	Induces cell proliferation, inhibits apoptosis	[39, 88, 89]
KGF/FGF-7	Keratinocyte Growth Factor/Fibroblast Growth Factor 7	Induces cell proliferation	[38, 42]
VEGF	Vascular Endothelial Derived Growth Factor	Induces angiogenesis, promote progenitor cell mobilization, inhibits apoptosis	[16, 37, 39, 41, 42, 87, 90]
Abbreviation	Full Name	Functions	
Anti-inflammatory cytokines			
TGF-β1	Transforming Growth Factor β	Induces stem cell differentiation, reduces inflammation/ immune activation	[38, 42, 88, 91]
IL-6	Interleukin 6	Stimulates stem/progenitor cell proliferation, induces angiogenesis	[16, 40, 41, 45, 49, 89, 90]
IL-10, IL-27, IL-17E, IL-13, IL-12p70, and (IL-1ra)	Interleukin 10, Interleukin 27, Interleukin 17, Interleukin 13, Interleukin 1 receptor antagonist	Anti-inflammatory	[38]
Pro-inflammatory cytokines			
IL-8	Interleukin 8	-Involves in mitogenesis -Inhibits angiogenesis, inflammation, chemotaxis, neutrophil degranulation, leukocyte activation, and calcium homeostasis.	[40, 41, 87]
IL-9	Interleukin 9	Stimulates cell proliferation and prevents apoptosis.	[41, 92]
IL-1b	Interleukin-1b	suppresses inflammation	[38]
Peptide & Hormones			
Ang	Angiopoietin	Induces angiogenesis and promotes cell survival	[87]
BMP	Bone Morphogenetic Protein	Regulates tissue homeostasis, promotes neurogenesis, induces stromal cell proliferation and migration, promotes angiogenesis	[88]
CNTF	Ciliary Neurotrophic Factor	Promotes survival of neurons	[93]
EPO	Erythropoietin	Induces angiogenesis, inhibits apoptosis	[94]
Gal	Galectins	Suppresses inflammation, induces stem cell mobilization, inhibits immune cell proliferation	[95, 96]

Table 3 (continued)

Abbreviation	Full name	Functions	Reference
GDNF	Glial cell line-Derived Neurotraphic Factor	Promotes survival of neurons, induces axonal growth, reduces infarct size	[94]
G-CSF	Granulocyte-Colony Stimulating Factor	Induces stem/progenitor cell proliferation, promotes neuronal differentiation	[39, 42]
GM-CSF	Granulocyte Macrophage- Colony Stimulating Factor	Stimulates stem cells to produce granulocytes and monocytes, plays a role in embryonic development, as a vaccine adjuvant in HIV-infected patients	[39, 41, 42]
M-CSF	Macrophage- Colony Stimulating Factor	Induces the proliferation, differentiation, and survival of monocytes, macrophages, and bone marrow progenitor cells, Increases tumor cell cytotoxicity	[39]
HO-1	Heme Oxygenase-1	Promotes induction of regulatory T cells	[97]
IDO	Indoleamine 2,3-Dioxygenase	Induces regulatory T cells, inhibits T cell activation	[97, 98]
MCP-1	Monocyte Chemotactic Protein 1	Induces angiogenesis, induces MSC migration, inhibits apoptosis	[16]
MIF	Macrophage Migration Inhibitory Factor	Inhibits macrophage migration	[38, 40, 41, 90]
PGE*2*	Prostaglandin E2	Suppresses inflammation, inhibits immune cell proliferation	[99]
SCF	Stem Cell Factor	Induces stem/progenitor cell proliferation, promotes neuronal differentiation	[97, 100]
SDF-1	Stromal Cell-Derived Factor 1	Regulates progenitor cell mobilization	[16, 37, 38, 87]
TSG-6	(TNF)-Stimulated Gene-6	Suppresses immune activation	[101, 102]
LL37	Human cathelicidin antimicrobial peptide	Anti-bacterial effect of MSC, direct killing of microorganisms, chemotaxis and chemokine induction -Regulates inflammatory responses - Angiogenic, anti-apoptotic, and wound healing effects	[103, 104]
Leptin	–	Promotes adipogenesis and reduces osteogenesis	[105]
Estrogen	–	Involves in memory impairment, increases fat store, maintenance of vessel and skin, reduces bone resorption, increases bone formation, supports hormone-sensitive breast cancers, promotes lung function by supporting alveoli	[106]

identified most of them with a general name. “secretome”. It can be injected by several ways: 1) direct injection into damaged tissues 2) intravenously, 3) transdermal and 4) intramuscular. The different proteomic studies have shown that various agents are present in secretome [31, 63, 81–85]. In fact, if MSCs consider as “building blocks”, their cytokines, growth factors and hormones can be assumed as “workers”. Growth factors lead to the activation, stimulation, and mobilization of stem cells from their origin. If more specific growth factors are produced, it would be possible in order to regenerate the damaged tissue specifically. Table 3 shows some cytokines, growth factors and hormones which are secreted by MSCs and their potential functions for tissue regeneration/repair.

Cytokines, Growth and Soluble Factors Act as Immunomudulatory

MSCs interact with various lymphocytes. During the innate and acquired immune systems, MSCs are able to inhibit the activation of pro-inflammatory monocytes and macrophages using secreting soluble factors such as transforming growth factor-β (TGF- β), hepatocyte growth factor (HGF), nitric oxide (NO), heme oxygenase (HO), interleukin (IL)-6, prostaglandin E2 (PGE2), indoleamine 2, and 3-dioxygenase (IDO) [107, 108]. Based on preclinical studies, it has been shown that MSCs have suppressive effects on both adaptive and innate immunity systems [109, 110]. MSCs are able to inhibit the activation of pro-inflammatory monocytes and macrophages. Moreover, in the presence of MSCs and their soluble factors, monocytes and macrophages may acquire anti-immunosuppressive functions. Moreover, MSCs inhibit the differentiation of monocytes into fully matured dendritic cells (DCs). The tolerogenic DCs produce a high level of IL-10 and decrease the ability of stimulate allogeneic T-cell proliferation in a mixed lymphocyte reaction [111–118]. MSCs prevent the proliferation and cytotoxicity of natural killer cells (NKs) mainly through PGE2 and IDO productions. MSCs are also able to suppress T-cell proliferation through the secretion of various soluble factors and inhibit T-cell activation. The immune modulatory factors are summarized in fig. 3. In addition, extensive clinical trials dealing with the immunomudolatory contribution of MSC-derived growth factors/ cytokines have also been reported (http://clinicaltrials.gov). MSC-derived growth factors/ cytokines have been promising for treatment of graft versus host disease(GVHD) [119, 120], inflammatory and autoimmune diseases such as multiple sclerosis or Crohn’s disease, diabetes mellitus type I and systemic lupus erythematosus (SLE) [110]. Pretreatment of MSCs with IFN-γ resulted in preventing of GVHD [121, 122] .

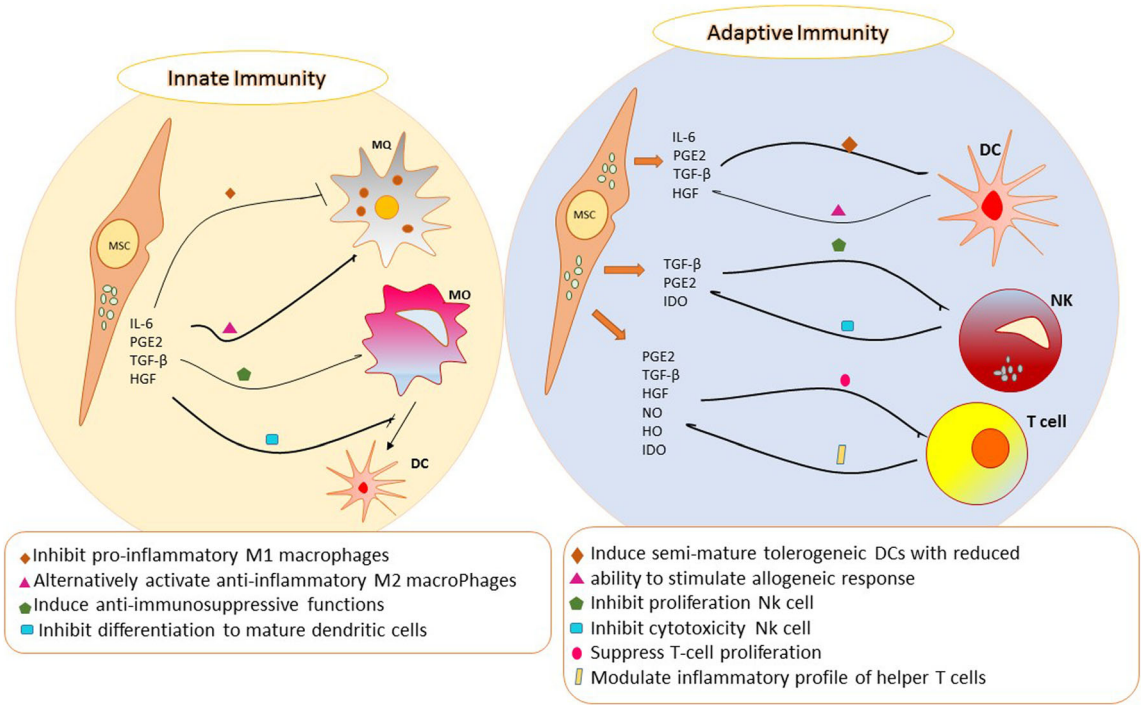

Fig. 3 Immunomodulatory properties of MSC-secretome. MSCs interact with various lymphocytes. During the innate and acquired immune systems, MSCs are able to inhibit the activation of pro-inflammatory monocytes and macrophages through the secretion of soluble factors. Additionally, MSCs inhibit the differentiation of monocytes into fully matured dendritic cells (DCs). MSCs prevent the proliferation and cytotoxicity of natural killer cells (NKs) and suppress the proliferation of the T cell. They also prevent the activation of the T cell through the cell to cell contact. MQ; Macrophage, MO; Monocytes, DC; Dendritic cells, NK; Natural killer

Table 4 Tissue- specific MSC-derived growth factors/cytokines implicated in regenerative medicine

Tissue/ disease	Growth factor/cytokine	Reference
Bone	- IGF-I - IGF-II - TGF-B - FGF - PDGF - PTHRP - BMP - GDF - …	[123–127]
Neutropenia	- G-CSF - GM-CSF - …	[128–130]
Wound healing	- EGF - TGF-B - HGF - VEGF - PDGF - FGF-I&II - TGF-α - KGF - …	[131, 132]
Cardiovascular & Myocardial Infarction	- VEGF - TIMP-2 - TSP-1 - TNF - IL-6 - IL-8 - MCP-I - HGF - FGF-2 - LIF - SCF - G-CSF - GM-CSF - EPO - IGF-1 - SDF-1 (SDF-1/CXCL12) - Ang-1 - TNF-α - …	[133, 134]
Liver	- HEGF - FGF-7 - EGF - HGF - TGF-B - IGF - SDF-1and receptor CXCR4 - VEGF	[38, 135]
Cardiovascular	- TGF-B - VEGF - MCP-1,3 - Ang-1 - NO - SDF-1a - CSF - GMCSF - TNF-a - IL-6	[136–142]

Table 5 Cytokines/growth factors approved by FDA

Cytokine/growth factor	Source	Clinical use	properties	Brand	Product Name
IFN-α	Genetically engineered *E. coli* strain / IFN-α from human leukocytes	-Hairy Cell Leukemia - Malignant Melanoma - Follicular Lymphoma - Condylomata Acuminata - Chronic Hepatitis C	For intramuscular, subcutaneous, intralesional, or intravenous injection	Roferon®-A	Interferon α-2a, recombinant
IL-2	Genetically engineered *E. coli* strain	-Metastatic renal cell cancer - Metastatic melanoma	For injection, intravenous infusion	PROLEUKIN® (aldesleukin)	Human recombinant interleukin-2
PDGF-BB	Yeast, *Saccharomyces cerevisiae*	Lower extremity diabetic neuropathic ulcers	For external use only (Rx Only)	REGRANEX (becaplermin) Gel	Recombinant human platelet-derived growth factor (rhPDGF-BB)
rh-GM-CSF	Yeast, Saccharomyces cerevisiae	- Acute Myelogenous Leukemia - Mobilization and Engraftment of PBPC (peripheral blood progenitor cells) - Autologous and allogeneic bone marrow transplantation	For injection, intravenous infusion (Rx Only)	Leukine (sargramostim)	Recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF)

Tissue- Specific MSC-derived Factors

The various factors may be presented into secretome as a cocktail and act in concert in order to promote regeneration. Therefore, it is important to analyze a complete set of growth factors and cytokine levels for every kind of stem cell-derived secretome/conditioned medium. While the content of the various cytokines in a certain secretome/conditioned medium is known, the potential outcome can be increased and translated to targeted therapy in regenerative medicine [45]. For example, bone regeneration could be occurred in the presence of insulin-like growth factor (IGF-I and IGF-II), transforming growth factor beta (TGF-β), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), parathyroid hormone-related peptide (PTHRP), bone morphogenic protein(BMP) into secretome/conditioned medium. Furthermore, the presence of epithelial growth factor (EGF), TGF- β, hepatocyte growth factor (HGF), vascular endothelial derived growth factor (VEGF), PDGF, FGF-I&II, TGF-α, and keratinocyte growth factor (KGF) play an important role in wound healing. Some of the specific growth factors and cytokines, which are implicated in the regeneration and repair of any tissue/disease, are listed in Table 4.

Growth Factors, Cytokines, and Hormones Available in the Market

The emergence of new classes of therapeutic agents which manufactured by biotechnology is one of the more exciting

Table 6 Mesenchymal stem cell recombinant growth factors

Product	Quality grade	Description	Source
Human FGF-1	Research grade	Recombinant human fibroblast growth factor 1	E. coli
Human FGF-2	Research grade	Recombinant human fibroblast growth factor 2	E. coli
Human BDNF	Research grade	Recombinant human brain-derived neurotrophic factor	E. coli
Human BMP-2	Research grade & Premium grade	Recombinant human bone morphogenetic protein 2	E. coli
Human BMP-4	Research grade & Premium grade	Recombinant human bone morphogenetic protein 4	Pichia pastoris
Human BMP-6	Research grade	Recombinant human bone morphogenetic protein 6	HEK293 cells
Human BMP-7	Research grade	Recombinant human bone morphogenetic protein 7	CHO cells
Human EG-VEGF	Research grade	Recombinant human endocrine gland-derived vascular endothelial growth factor	E. coli
Human EGF	Research grade & Premium grade	Recombinant human epidermal growth factor	E. coli
Human FGF-2 IS	research grade	Recombinant human fibroblast growth factor 2 IS (improved sequence)	
Human G-CSF	research grade premium grade	Recombinant human granulocyte colony-stimulating Factor	E. coli
Human Galectin-1	research grade	Recombinant human galectin 1	E. coli
Human GM-CSF	research grade premium grade	Recombinant human granulocyte macrophage colony–stimulating factor	E. coli
Human HGF	Research grade	Recombinant human hepatocyte growth factor	E. coli
Human IFN-α2a Human IFN-α2b	Research grade	Recombinant human interferon α2a Recombinant human interferon α2b	E. coli
Human IFN-β1a Human IFN-β1b	Research grade	Recombinant human interferon β1a Recombinant human interferon β1b	CHO cells E. coli
Human IFN-γ1b	Research grade	IFN-γ Recombinant human interferon γ1b	E. coli
Human IGF-1 Human IGF-2	Research grade	Recombinant human insulin-like growth factor 1 Recombinant human insulin-like growth factor 2	E. coli
Human M-CSF	Research grade	Recombinant human macrophage-colony stimulating Factor	E. coli
Human MCP-1	Research grade	Recombinant human monocyte chemotactic protein 1	E. coli
Human MIF	Research grade	Recombinant human macrophage migration inhibitory Factor	E. coli
Human SCF	Research grade premium grade	Recombinant human stem cell factor	E. coli
Human SDF-1α	Research grade	Recombinant human stromal cell-derived factor 1α	
Human TGF-β1 Human TGF-β2 Human TGF-β3	Premium grade Research grade	Recombinant human transforming growth factor β1	HEK293 cells Insect cells HEK293 cells
Human TNF-α	Premium grade Research grade	Recombinant human tumor necrosis factor α	E. coli Yeast
Human VEGF	Research grade	Recombinant human vascular endothelial growth factor	Insect cells

frontiers in medicine. The possibility of clinical usage of recombinant growth factors and cytokines expressed by MSCs has been proved; however, the application of that is limited. Reproducibility of the growth factors, cytokines, HLA incompatibility and the infectious agentstransmission possibility might be the reasons behind them. The emergence of MSC-derived growth factors and cytokines will address many of these problems and pave the way for its evaluation in a variety of diseases. A number of cytokines have already been licensed by the Food and Drug Administration (FDA) in clinical application (Table 5). The FDA must evaluate an ever-increasing number of new growth factors and cytokines. In order to develop regulatory policy for using of these products from laboratory bench to the bedside, several factors including those combined sound scientific principles and good clinical medicine should be considered, however, the final goal should be beneficial for patients. In order to achieve a successful cell-based clinical trial, high quality of raw materials is essential. Interestingly, the MSCs inherently express many growth factors and cytokines with the highest degree of good manufacturing practice (GMP). Therefore, harvesting these therapeutic agents from MSCs would be an important part of the stem cell research industry in perspective of natural bioreactor and producer cells. Table 6 summarizes some of these growth factors and cytokines which produced by several companies that are used for laboratory and research purposes. It is noteworthy that the GMP of MSCs-derived growth factors and cytokines are different from those stem cells that transplanted to patients. When MSC-derived growth factors and cytokines are packaged properly, they can be transported easily like other drugs and they do not need cryopreservation. However, in comparison with recombinant growth factors that may be stable for a long period of time and also produced on a larger scale in non-stem cell hosts, MSC-derived growth factors and cytokines which need more optimization in terms of production and stability. A number of growth factors and cytokines in the secretome which have been expressed separately in MSCs using recombinant DNA technology are presented in Table 7. For clinical application, a large amount of growth factors/cytokines are needed. Hence, manufacturing large quantities (scale-up) of hMSC's secretome based on GMP-procedures will be challenging.

For clinical application, it is essential to improve the production of MSC-derived growth factors/ cytokines. A variety of biotechnological techniques such as suspension culture, cultivating with three-dimensional (3D) scaffolds, cultivating with an advanced bioreactor, cultivating under sublethal doses of oxidative stress, hypoxia and magnetic field (MF) can be employed in this area [16, 143]. The aims of aforementioned techniques are to simulate and reproduce the stem cell niche in order to improve MSCs quality and in turn MSC-derived growth factors/cytokines. In addition, genetically modified MSCs with cytoprotective factors such as nuclear factor-erythroid 2-related factor 2 (Nrf2), TGFβ1, HO-1, lipocalin 2 (Lcn2), VEGF, hypoxia-inducible factor (HIF-1α), IGF-1 and etc. could be other strategies to improve MSC-derived growth factors/ cytokines [16–18, 76].

Table 7 A number of growth factors and cytokines in the secretome which have separately been expressed in MSCs by recombinant DNA technology

GMP Grade MSC Agents	
GMP Recombinant Human FGF-2	GMP Recombinant Human IL-2
GMP Recombinant Human Flt3-ligand	GMP Recombinant Human IL-3
GMP Recombinant Human GM-CSF	GMP Recombinant Human IL-4
GMP Recombinant Human GM-CSF	GMP Recombinant Human IL-6
GMP Recombinant Human SCF	GMP Recombinant Human IL-7
GMP Recombinant Human TNF-α	GMP Recombinant Human IL-15
GMP Recombinant Human IL-1β	GMP Recombinant Human IL-21

Conclusion

In clinical perspective, MSCs have drawn lots of interests for more than one decade. However, concerns about tumor formation and low survival rate after transplantation are the main limitations that impair their widespread usage in clinic. Instead, a number of studies have shown that MSCs can exert their therapeutic roles via producing of a vast array of bioactive molecules such as growth factors, cytokines, peptides, hormones, and microRNAs. These unique properties of MSCs are convincing to call MSCs as an arsenal of therapeutic agents. In other words, MSCs naturally and innately act as a bioreactor in order to produce a large number of valuable pharmaceutical products as well as, open a new horizon for basic, clinical scientists and marketing.

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Compliance with Ethical Standards

Conflict of Interest The authors declare there is no conflict of interest.

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