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Therapeutic uses of post-partum tissue-derived mesenchymal stromal cell secretome

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Human post-partum tissue mesenchymal stromal cells (hPPT-MSCs) are widely used in research to investigate their differentiation capabilities and therapeutic effects as potential agents in cell-based therapy. This is ascribed to the advantages offered by the use of MSCs isolated from hPPT over other MSC sources. A paradigm shift in related research is evident that focuses on the secretome of the human MSCs (hMSCs), as therapeutic effects of hMSCs are attributed more so to their secreted growth factors, cytokines and chemokines and to the extracellular vesicles (EVs), all of which are components of the hMSC secretome. Positive therapeutic effects of the hPPT-MSC secretome have been demonstrated in diseases related to skin, kidney, heart, nervous system, cartilage and bones, that have aided fast recovery by replacing damaged, non-functional tissues, via differentiating and regenerating cells. Although certain limitations such as short half -life of the secretome components and irregular secreting patterns exist in secretome therapy, these issues are successfully addressed with the use of cutting-edge technologies such as genome editing and recombinant cytokine treatment. If the current limitations can be successfully overcome, the hPPT-MSC secretome including its EVs may be developed into a cost-effective therapeutic agent amenable to be used against a wide range of diseases/disorders.

Key words Extracellular vesicles - human mesenchymal stromal cell secretome - post-partum tissue - stem cell therapy

Introduction

Stem cell (SC) research has brought regenerative medicine to the forefront of cell-based clinical research and therapy due to promising outcomes. SC research has produced cells, tissues and whole organ-like structures *in vitro* using SCs to replace damaged or non-functional tissues or organs by transplantation[1]. Diseases such as autism[2] and muscular dystrophies[3] which are labelled as incurable have gained promising results using SC therapy.

Pluripotent embryonic SCs (ESCs) isolated from the blastocyst stage of embryos have ethical concerns than the multipotent adult SCs which are isolated from the bone marrow, brain and heart tissue; post-partum tissue (PPT) such as umbilical cord (UC), UC blood (UCB), amniotic membrane (AM) and placenta and surgical waste such as deciduous teeth pulp and adipose tissue (AD)[4]. As ESC sources are the stored embryos at assisted reproductive centres, varying ethical issues dependent on the country of use, in addition to their tumourigenic capacity have limited the use of ESCs in

research[5]. Induced pluripotent SCs, adult cells which are reprogrammed to function as embryonic-like SCs, have also shown great potential in therapeutics[6]. Among the different types of adult SCs, bone marrow SCs (BM-SCs) have widely been used in research. With the ability of establishing SCs from biological and surgical waste, SC research has flourished mainly due to the minimum ethical considerations associated with the use of such waste starting material[7]. High availability[8], absence of tumourigenicity and favourable immune-privileged effects[9] are other reasons for the increased use of SCs in biological waste material to fulfil the demand of the ever-rising numbers of therapy trials. Mesenchymal stromal cells (MSCs) and haematopoietic SCs (HSCs) derived from PPT are such adult SC categories that are in the initial stages of clinical trials. As at November 2020, the US National Institutes of Health SC registry lists 5685 SC-related clinical trials, of which 86 are related to HSCs derived from cord blood and 58 trials related to MSCs from other PPTs[10].

With the use of relatively easy isolation methods, low rejection rates, high availability and wide differentiation potential, PPT-SCs have gained attention in SC research. hMSCs can be isolated from all PPTs using either digestion or explant methods. UCB provides a source to isolate HSCs by selecting CD34+ cells and expanding them in a suitable medium supplemented with selected cytokines as non-adherent cultures[11] or else UCB can be used to isolate MSCs by expanding mononuclear cells (MNCs) as adherent cultures[12]. UCB-hMSC showed significantly higher proliferation, clonality and/or significantly lower expression of p53, p21 and p16, well known markers of senescence, compared to BM and AD hMSCs[11]. However, successful isolation of hMSCs from UCB is believed to depend on the time between collection and isolation, the net volume of blood and the MNC count; hence, the isolation process itself becomes laborious and time-consuming, resulting in low yields of hMSCs[13]. Due to such difficulties confronted, as well as contemplation by parents on storing UCB in blood banks for future use of their child, the use of other types of PPT are considered. Although their self-renewal capacity when compared with other hMSCs was not significantly different, placenta-hMSC showed high proliferative capacity and better growth characteristics than bone marrow, adipose-derived and UCB-hMSCs[11]. Expression of stemness markers was not significantly different between these hMSCs, making UCB and placenta-hMSCs potential candidates for research akin to bone marrow- or adipose-derived hMSCs[11]. Although 5-50 per cent of SC marker-positive cells reside within the population of amniotic epithelial cells, a mere 0.01-0.1 per cent SCs are present within the other residing tissue types[14], making the AM a rich source of SCs compared to somatic tissues. Human AM-MSCs, UC-MSCs and UCB-MSCs also demonstrate immunosuppressive properties[15-17]. In an *in vitro* study, human placenta-MSCs have shown a significantly higher ability of immunosuppression compared to human UC-MSCs[18].

It is believed that most of the therapeutic effects are due to different bioactive molecules such as growth factors, cytokines, chemokines and angiogenic factors that are secreted by SCs which are collectively known as the 'stem cell secretome', and all these molecules have been thoroughly investigated[19]. It is reported that the UC-MSC secretome is significantly different from the bone marrow- and adipose-derived SC secretomes[20]. This article reviews the therapeutic effects of the UC-derived mesenchymal SC secretome and highlights the pros and cons of its applications, compared to other SC secretomes.

Composition of the PPT-MSC secretome

A typical hMSC secretome is known to contain growth factors, cytokines, extracellular vesicles, lipid mediators, extracellular membrane proteases and hormones[21], causing differential effects on the treated cells.

Growth factors and cytokines

Composition of the Wharton's jelly (WJ) hMSC secretome was investigated using homonuclear magnetic resonance and multiplexing laser bead technology in a study, where it was discovered that compared to unconditioned medium, conditioned medium consisted of increased levels of transforming growth factor-β1, epidermal growth factor (EGF), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor-AA and vascular endothelial growth factor (VEGF) as well as a range of cytokines such as interleukin (IL)-12p70, interferon-gamma, IL-17A and IL-10[22]. VEGF and fibroblast growth factor (FGF) possess cardioprotective and cardioregenerative effects[23]; the inclusion of VEGF in the UC-hMSC secretome may lead the secretome to manifest such properties. Wound healing capacity of the secretome may be attributed to the presence of IL-6, IL-8 and MCP-1

that enhance monocyte migration into injured sites, thereby suggesting the migration of other cell types such as fibroblasts into the wound sites with the help of mentioned cytokines when treated with the secretome[24]. There is a wide range of proliferative and anti-apoptotic growth factors, immunomodulatory, immunosuppressive cytokines and chemokines listed as constituents of the secretome[22], which may well surmount to different activities and effects exerted by the secretome.

Extracellular vesicles (EVs)

Other than the soluble factors of the secretome, extracellular vesicle (EV) is an additional distinct component with a size range of 80 nm to 1 µm[25] and categorized into three subtypes: exosomes, microvesicles and apoptotic bodies[26]. Components such as proteins, lipids and functional genetic material [DNA, microRNA (mRNA) and fragmented DNA] present in these vesicles are transferred into other target cells aiding regulation requirements for therapeutic procedures in SC therapy[27]. UC-MSC-derived nanovesicles have been reported to confer therapeutic effects on skin burn rat models by accelerating skin damage repair via Wnt-signalling pathway[28] and murine models on hypoxic pulmonary hypertension by exerting lung protection and reducing pulmonary hypertension via STAT-3-mediated signalling pathway[29]. Both these studies report that the exosome-carrying EVs are responsible for the therapeutic effects, suggesting mRNA-mediated cell signalling.

Large-scale manufacturing of EVs is required to be used in therapeutic platforms. Different culture systems with varying parameters such as thermal stress, hypoxia, radiation, increase of intracellular calcium levels and sulphydryl-blocking agents have been identified as potential factors which enhance the EV-secreting ability[30].

Significance of the hPPT-MSC secretome

Of the MSC secretomes, the UC-MSC secretome has proven to be significantly different from BM-MSC and adipose-derived MSC secretomes[31]. UC-MSCs show significantly reduced synthesis of important proangiogenic factors but increased secretion of angiogenic growth factors and chemokines when compared to BM-MSCs and AD-MSCs[32-34]. UC-MSCs have also demonstrated significantly higher increased secretion of neurotrophic factors[35], important cytokines and haematopoietic growth factors than the BM-MSC and AD-MSC secretomes[36], pointing towards the potential benefits of therapy specific to the UC-MSC secretome. A study comparing the effects of BM-MSC and WJ-MSC secretomes on neural differentiation demonstrated different temporal profiles regarding stimulation of neurite outgrowth and the gene expression of neuronal markers[37]. Although proteomic-based mass spectrometry has shown differences of protein profiles among the BM, AD and UC-MSC secretomes, it also confirmed that the UC-MSC secretome is a potential candidate for neuroregenerative research as much as the other two secretomes[31]. UC-MSCs cultured using post-partum waste and the resultant secretome obtained with ease, together with minimum ethical considerations, will augment its value in cell-free therapeutic procedures. The following subsections highlight research in which UC-MSC secretome was investigated in different therapeutic procedures against a wide range of diseases.

Therapeutic effects of hPPT-MSC secretome

Anti-ageing and other skin repair therapies

Skin is the main target of most cosmetic products. Anti-ageing and skin tone-lightning products are enormously marketed by pharmaceutical and cosmetic companies. Importance of using naturally derived stimulants or inhibitors for cosmetic purposes is highly recommended as skin is a very sensitive organ and the consumers are extremely cautious about the side effects and toxicity of such products. Pharmaceutical companies are focusing on naturally derived components to reduce their production costs which may target a wide array of the population regardless of their economic status.

Late recovery of skin wounds caused by different injuries is also a growing concern as it decreases the quality of life of the patient by scar formation and increased risk of infection[38]. Diabetic wounds result only in 50 per cent short-term recovery, even under high standard treatment methods[39], which suggests that the current therapeutic methods require change. Conversely, burn wounds also require critical care to stabilize and functionally recover the patients[40]. Table I lists the research where PPT-derived SC-conditioned medium was used to manifest anti-ageing and anti-melanogenesis effects, as well as to successfully recover wounds of different origin, *i.e.,* diabetic wounds and burn wounds. Type of animal model or human cell lines used for these

Table I. Use of human post-partum tissue mesenchymal stromal cells secretome in treating diseases related to skin

Type of secretome/CM	Disease	Animal models or cell type	Outcome	Possible mechanisms	References
WJ-hMSC	Ageing effects	UVA irradiated human dermal fibroblasts (*in vitro*)	Increased proliferation, migration rates and TGF-β signalling[41]	Increased cell migration via TGF-β smad signalling pathway	Sánchez-A, 2005[42]
UC-hMSC	Ageing effects	Human dermal fibroblasts in high glucose induced diabetic microenvironment (*in vitro*)	Decreased ROS production and senescence	Antioxidant and anti-ageing effects through downregulating expression of senescence-related genes	Li *et al*, 2017[43]
UC-hMSC Subcutaneous administration into animal model	Diabetic wounds	Delayed wound healing mouse models (diabetic wounds) (*in vivo*)	Significantly higher wound closure rates, capillary densities and PDGF-β, KGF, VEGF expression levels	By increasing expression of important growth factors related to dermal healing	Shrestha *et al*, 2013[44]
UC-hMSC Burn wounds were topically treated	Burn wounds	Rats with induced burn wounds (*in vivo*)	Acceleration of wound closure. High density of collagen fibers, increased numbers of fibroblasts and blood vessels	bFGF-mediated cell regeneration	Padeta *et al*, 2017[45]
Hypoxic AF-hMSC Subcutaneous administration into animal model	Skin wounds	Rats with induced wounds (*in vivo*) and human skin fibroblasts (*in vitro*)	Enhanced proliferation and migration of human dermal fibroblasts *in vitro* and wound healing in rat model	Via TGF-β/SMAD2 and PI3K-PKB/Akt pathways	Jun *et al*, 2014[46]
UC-hMSC Wounds were topically treated	Wound healing	Human umbilical vein endothelial cells (*in vitro*) and rats with induced wounds (*in vivo*)	Decreased inflammation at initial stage, cell migration and angiogenesis stimulation *in vitro* and *in vivo*	-	Kusindarta *et al*, 2016[47]
UC-hMSC infected with Wnt7a-expressing virus Subcutaneous administration into animal model	Cutaneous wounds	Mice with full thickness skin injury (*in vivo*)	Stimulation of wound closure and regeneration of hair follicles	Via activating fibroblasts enhanced secretory expression of ECM components which promotes keratinocyte migration and reepidermalization. Also enhances crosstalk between cells in complex wound microenvironment	Dong *et al*, 2017[48]
UCB-hMSC	Melanin synthesis (cosmetic use)	Hyperpigmented melanoma cells and normal human epidermal melanocytes (*in vitro*)	Inhibition of melanogenesis	Via degradation of MITF expression via the ERK signalling pathway	Kim *et al*, 2015[49]
UCB-hMSC Topical treatment with CM on wrinkle sites	Skin ageing	Dermal fibroblasts and women with wrinkles	Stimulate growth and production of HDFs by ECM, promoted antiwrinkle effect and dermal density was significantly increased in women	GDF-11 aided in promoting skin rejuvenation via upturned growth and ECM production of human dermal fibroblasts	Kim *et al*, 2018[50]

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Type of secretome/CM	Disease	Animal models or cell type	Outcome	Possible mechanisms	References
WJ-hMSC	Radioactive dermatitis	UVEC and rat models with radiation induced skin wounds	Stimulated proliferation of UVECs, sebaceous glands were regenerated and stimulated angiogenesis and wound healing *in vivo*	-	Sun *et al*, 2019[51]

CM, conditioned medium; ECM, extracellular matrix; MITF, microphthalmia-associated transcription factor; HDFs, human dermal fibroblasts; ECM, extracellular matrix; GDF-11, growth differentiation factor-11; UVEC, umbilical vein endothelial cells; TGF- β, transforming growth factor beta; ROS, reactive oxygen species; hMSC, human mesenchymal stromal cells; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; KGF, keratinocyte growth factor; BFGF, basic fibroblast growth factor; PI3K, phosphoinositol-3-kinase; WJ, Wharton's jelly; UCB, umbilical cord blood; UC, umbilical cord; AF, amniotic fluid; ERK, extracellular signal-regulated kinase; UVA, ultraviolet A, ; PKB, protein kinase B

experiments, the outcomes and plausible underlying mechanisms of each report are also summarized in Table I.

Anticancer therapy and other cancer-related therapy

As projected in 2012, by 2030, of the global cancer burden, new cancer cases will account to 21.7 million and cancer deaths are calculated around 13 million[52]. Despite cancer screening programmes for early detection, public awareness programmes and treatment methods linked with novel technological advances, cancer had struck globally with no impact of the economic status of the countries[53] Effective treatment is a major component of a balanced approach to cancer[54], where anticancer drugs and other methods to eliminate cancer are investigated to match the ever-rising numbers of cancer patients and different cancer types. Of the small molecules approved as anti-cancer drugs from 1940s to 2012, 48.5 per cent were reported to be natural products or derivatives of natural products[54]. However, cytokines, growth factors and other compounds extracted from human biological material appear to be equally effective in anticancer therapy; hence, the hMSC secretome was investigated for its anticancer potential. Table II elaborates the use of human PPT (hPPT)-MSC secretome on anticancer-related therapy for human laryngeal carcinoma, lung cancers, leukaemia, hepatic and cervical cancers investigated in *in vitro* studies using human cancer cell lines. The outcomes were apoptosis, inhibition of drug resistant effects, antiproliferative and cell viability effects; the associated mechanisms are listed in Table II. In addition, Zimmerlin *et al*[61], reported on many MSC-secreted factors effective on a wide range of cancers including non-specified paracrine factors secreted from UC-MSCs.

Use of hPPT-MSC secretome in therapeutic procedures against various other diseases

In vitro differentiated cells have the advantage of aiding fast recovery of the patient, rather than the time-consuming method of transplanting undifferentiated SCs which would differentiate and then replace the non-functional or injured tissue. Procedures to differentiate SCs into a variety of mature cell types *in vitro* and *in vivo* under different stimulated conditions such as by adding synthetic and natural compounds have been investigated; and, the hMSC secretome rich in various growth factors and cytokines has also been explored. In 2014, a phase 1 clinical trial was set up with 20 patients, to investigate the

Table II. Use of human post-partum tissue mesenchymal stromal cells secretome on anticancer therapy

Type of secretome/CM	Type of cancer	Human cell line used	Outcome	Possible mechanisms	References
WJ-hMSC	Human laryngeal carcinoma	Hep-2 cell line (*in vitro*)	Caused apoptosis in Hep-2 cells	Via increase p53 and decrease of Bcl-2	Elias *et al*, 2016[55]
WJ-hMSC	Lung cancer	A549 lung cancer cells (*in vitro*)	Drug resistant effects inhibited	-	Hendijani *et al*, 2015[56]
WJ-hMSC	Leukaemia cells	K562 leukaemia cells	Significant antiproliferative effects	-	Hendijani *et al*, 2014[57]
AM-hMSC	Hepatic carcinoma	HepG2 cell line (*in vitro*)	Decreased cell proliferation and cell viability	Via increased expression of p53, p21 and Caspase 3 (proapoptotic mRNA) and diminished expression of Ki-67 (cell proliferation marker)	Riedel *et al*, 2017[58]
UCB-hMSC	Cervical cancer	HeLa cell line (*in vitro*)	Significantly induced apoptosis	Via mitochondrial apoptotic pathway	Sandra *et al*, 2014[59]
WJ-MSC	Breast Cancer	MCF-7 cell line (*in vitro*)	Induced apoptosis	Not specified	Mirabdollahi *et al*, 2019[60]

CM, conditioned medium; WJ, Wharton's jelly; hMSC, human mesenchymal stromal cells; AM, amniotic membrane; UCB, umbilical cord blood; mRNA, microRNA

effect of microvesicles derived from UCB-MSCs, to decrease the inflammatory state and enhance the β-cell mass as well as the glycaemic control[62]. Two recent studies have also been registered on NIH clinical trials, US data base, on uses of secretome of adipose derived MSC for the treatment of Osteoarthritis and for Articular Regeneration and using hypoxia-MSC secretome to treat COVID-19 patients[63,64]. Table III summarizes such research where the secretome was used in cell differentiation and cell protection protocols for therapeutic applications in cartilage disorders, Parkinson's disease, ischaemia, cardiotoxicity, acute myocardial infarction, pulmonary artery hypertension, chronic renal disease and skeletal muscle atrophy.

In addition, HSCs were also reported to increase their proliferation rates due to paracrine factors secreted by WJ-hMSCs such as IL1a, IL-6, IL-7, IL-8 cytokines, hyaluronic acid, cell adhesion molecules, cadherins and growth factors [stem cell factor and hepatocyte growth factor (HGF)] which are secreted in high amounts than BM-hMSC[36,77]. Figure presents the gist of the review in a nutshell.

Limitations and the way forward with the hPPT-MSC secretome

Although many potential beneficial therapeutic advantages of the PPT-MSC secretome are apparent, yet the most important issue involved is controlling the MSCs to continuously secrete the required factors in adequate amounts, because the secretion of secretome factors varies due to the state of the cells and the passage number of the cell line[78,79]. In therapeutic procedures where hPPT-MSCs are transplanted, the short half-life of the secreted factors, such as HGF which only remains viable for 3-5 min, raises another concern; administering continuous doses of such secreted stimulants with extremely short half-lives to patients is required for positive therapeutic effects[80]. Solution to this problem was provided with the use of genome-editing technologies, where the genome of UCB-hMSCs was edited to render the cells continuously secrete HGF, but in an induced manner[76]. Furthermore, treatment of hMSCs with different cytokine cocktails modified the hMSC secretome by directing hMSCs to secrete specific required factors to render considerable therapeutic effects against, for example, liver inflammation by improving the immunomodulatory capacity[81].

Existing literature supporting the presence of the therapeutic effects of EVs is another future aspect of

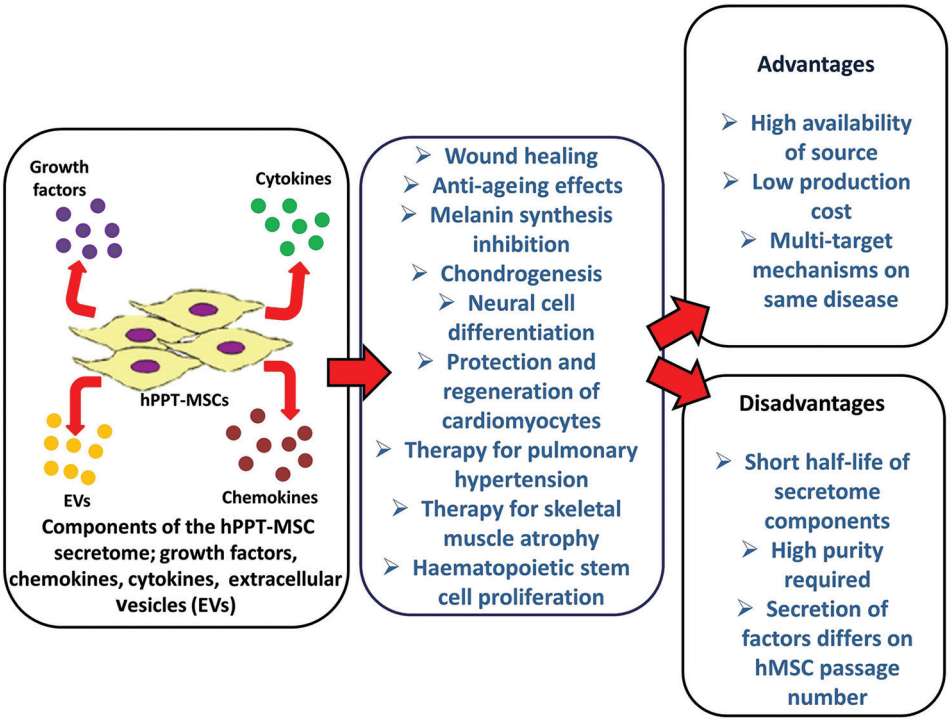

Figure. Human post-partum tissue mesenchymal stromal cells secrete growth factors, chemokines, cytokines and extracellular vesicles which form the components of its secretome. These secretome components manifest different therapeutic effects. List of advantages and disadvantages of the therapeutic use of human post-partum tissue mesenchymal stromal cell secretome over current cell therapy methods is provided.

the hMSC secretome to be examined. Purification of these EVs from the secretome is important as a study demonstrated that the purity of EVS secreted by UC-hMSCs is a limiting factor for their immunosuppressive effects[82]. Provision of solutions to issues related to the therapeutic uses of MSC secretome by means of gene editing, cytokine therapy and extrapurification procedures may however, lead to increased charges of such therapeutics, rendering these unavailable for the developing world. This single reason could mar the beneficial use of the hMSC secretome or its secreted components in therapy; hence, when producing at the commercial scale, it is crucial to adapt to procedures where the current limitations will be overcome in a cost-effective manner. Furthermore, the mRNAs transported by exosomes had been reported to be mediators of cancer communication and also associated with a number of neurodegeneration disorders; hence, further analysis of these derivatives should be done before clinical applications[83]. Use of standardized herbal extracts as an alternative may be an inexpensive option as a range of herbal extracts have shown proliferation and differentiation abilities when used on SCs[7], suggestive of induced changes to the secretome. Countries rich in biodiversity and traditional medicine knowledge can actively contribute to achieve this goal, collaborating with countries which possess cutting edge technological advances, so that 'induced secretome therapy' may be affordable globally.

Conclusions

The properties of the hPPT-MSC secretome, provided through a strong and growing body of evidence, bear ample testimony to the potential therapeutic usage of it. However, extensive clinical trials are warranted to reinforce facts and figures obtained by *in vitro* and *in vivo* animal studies. Limiting factors of the hPPT-MSC secretome in therapeutic usage can be surmounted by strategies with the help of cutting-edge technologies. However, there is a risk in decreasing the cost-effectiveness of the proposed secretome therapy by the use of such novel technological advances using expensive reagents and equipment; hence, as an alternative, standardized herbal extracts may be used which are naturally available, cheaper, non-toxic and scientifically proven and are effective on hMSCs that will render these cells and their secretome therapeutically feasible, by inducing hMSCs to secrete its components selectively,

Table III. Use of human post-partum tissue mesenchymal stromal cells secretome in cell differentiation, cell protection and various other disease therapeutics

Type of secretome/CM	Therapeutic application	Animal models or cell type	Outcome	Possible mechanisms	References
Thrombospondin-2 secreted by UCB-hMSC	Cartilage disorders	Chondro progenitor cells and rabbits with full-thickness osteochondral defects	Increased chondrogenic effects	Through signalling pathways such as PKCα, ERK, p38/MAPK and notch	Jeong *et al*, 2013[65]
WJ-hMSC	Cartilage disorders	Chondrocytes	Increased expression of cartilage specific genes	Via significantly enhanced expression of collagen type II, Sox-9, aggrecan and COMP genes	Hassan Famian *et al*, 2017[66]
Amniotic epithelial cells	Dopaminergic neuron to treat Parkinson's disease	UCB-hMSC	Differentiation into dopaminergic neuron-like cells	Through neurotrophic factor BDNF and NGF, derived in brain	Yang *et al*, 2013[67]
UCB-hMSC	Protection of ischaemic cardiomyocytes	Murine HL-1 cardiomyocytes subjected to stimulated ischaemia	Decreased number of dead cells and increased viability	Via enhancement of Akt, ERK and transcription factor STAT3 (cell survival promoting kinases) phosphorylation	Bader *et al*, 2013[68]
Amniotic fluid SC	Protection from cardiotoxicity	H9c2 cardio myoblasts and primary mouse neonatal ventricular cardiomyocytes	Blockage doxorubicilin induced cardiotoxicity senescence and apoptosis	Via activation of PI3K/ Akt signalling cascade and upregulation of its related genes	Lazzarini *et al*, 2016[69]
AM-hMSCs Injecting CM into infarcted rat hearts	Acute myocardial infarction	Rat models with heart infarcts	Infarct size limitation, reduced cardiomyocyte apoptosis, ventricular remodeling and increased capillary formation	Via activation of prosurvival ERK1/2 MAPK pathway and inhibition of SAPK/ JNK and p38 MAPK proapoptotic pathways[68]	Danieli *et al*, 2015[70]
UCB-hMSCs Infused CM into rat models via tail vein	PAH	monocrotaline induced PAH rat model	Reduced ventricular pressure, the right ventricle/(left ventricle + interventricular septum) ratio and respiratory functions properly managed	Via enhanced IL-1α, CCL5 and TIMP-1 levels	Lee *et al*, 2016[71]
UC-hMSC Prior to administration of CM via the left renal artery, total ligation of the left ureter was done	Chronic renal disease	Rat model with unilateral ureteral obstruction	Positive treatment of renal interstitial fibrosis	Via significant reduction of MDA and ROS and enhanced activity of GSH	Liu *et al*, 2017[72]

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Type of secretome/CM	Therapeutic application	Animal models or cell type	Outcome	Possible mechanisms	References
UC-hMSC Soleus muscles of both hind legs were injected with CM	Skeletal muscle atrophy	Hind limb muscle atrophy models	Significantly improved muscle mass and muscle fiber size	Via enhancing the PI3K-PKB/Akt signalling cascade	Kim *et al* 2016[73]
UC-hMSC	Irradiation myocardial fibrosis	Irradiated primary HCF	Improved cell viability, reduced collagen deposition, prevented oxidative stress, increased antioxidant status and reduced pro-fibrotic cytokines	Via inhibition of the NF-κB signalling pathway	Chen *et al*, 2018[74]
UC-hMSC Administered via left renal artery	Renal fibrosis	Rat models with renal interstitial fibrosis	Decreased deposition of extracellular matrix, inflammatory cell infiltration and release of inflammatory factors	Via inhibiting TLR4/ NF-κB signalling pathway activation	Liu *et al*, 2018[75]
Extra cellular vesicles of Adipose derived MSC Intravenous administration	Autoimmune Encephalomyelitis (AE)	Mice models with induced experimental (AE)	Reducing proliferative potency of T cells, leukocyte infiltration, and demyelination	Not reported	Jafarinia *et al*, 2020[76]

BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; PI3K, phosphoinositol-3-kinase; SC, stem cell; PAH, pulmonary artery hypertension; HCF, human cardiac fibroblasts; MDA, malondialdehyde; ROS, reactive oxygen species; GSH, glutathione; hMSC, human mesenchymal stromal cells; UCB, umbilical cord blood; UC, umbilical cord; AM, amniotic membrane ; PKCα, Protein Kinase C-alpha ; ERK, extracellular signal-regulated kinase; p38/MAPK, p38 mitogen-activated protein kinases; TLR4/NF-κB, Toll-like receptor 4/nuclear transcription factor-κB; PKB, Protein Kinase B

in a continuous manner. If the current limitations posed may be successfully overcome, the secretome of PPT MSCs inclusive of its EVs may become an effective therapeutic agent which could be used against a wide range of diseases/disorders.

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