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Review Article

Biological activity of mesenchymal stem cells secretome as a basis for cell-free therapeutic approach

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Abstract

Mesenchymal stem (stromal) cells (MSCs) are self-renewing, cultured adult stem cells which secrete a complex set of multiple soluble biologically active molecules such as chemokines, and cytokines, cell adhesion molecules, lipid mediators, interleukins (IL), growth factors (GFs), hormones, micro RNAs (miRNAs), long non-coding RNAs (lncRNAs), messenger RNAs (mRNAs), exosomes, as well as microvesicles, the secretome. MSCs of various origin, including adipose-derived stem cells (ASCs), bone marrow derived mesenchymal stem cells (BM-MSCs), human uterine cervical stem cells (hUCESCs), may be good candidates for obtaining secretome-derived products. Different population of MSCs can secret different factors which could have anti-inflammatory, anti-apoptotic, anti-fibrotic activities, a neuroprotective effect, could improve bone, muscle, liver regeneration and wound healing. Therefore, the paracrine activity of conditioned medium obtained when cultivating MSCs, due to a plethora of bioactive factors, was assumed to have the most prominent cell-free therapeutic impact and can serve as a better option in the field of regenerative medicine in future.

Keywords

mesenchymal stem cells; pre-conditioning; secretome; regeneration; immunomodulation; therapeutic potential.

Introduction

Mesenchymal stem (stromal) cells (MSCs) are self-renewing, culture expandable adult stem cells that have been shown to be a promising candidate for cell-based therapy (Ferreira et al. 2018). Using mesenchymal stem cells for regenerative purposes is possible due to their trophic, paracrine, and immunomodulatory properties (Stagg and Galipeau 2013; Del Papa et al. 2015; Marfia et al. 2015; Leavitt et al. 2016; Del Papa et al. 2019; Kucharzewski et al. 2019; L et al. 2019; Lombardi et al. 2019). Besides, MSCs also have anti-tumorigenic, anti-fibrotic, anti-apoptotic, anti-inflammatory, pro-angiogenic, neuroprotective, anti-bacterial and chemo-attractive effects (Maumus et al. 2013; Bartosh et al. 2016; L et al. 2019).

MSCs harvested from numerous anatomical locations, including the bone marrow, adipose tissue, Wharton's jelly of the umbilical cord, display similar immunopheno-

typic profiles. However, there is a large body of evidence showing that, despite the similarity in their immunophenotypes, MSCs secrete a complex set of multiple soluble biologically active molecules, the secretome, composition of which varies significantly, depending on the age of the host and niches where the cells reside (Baksh et al. 2004; Traktuev et al. 2008; Amos et al. 2010; Daquinag et al. 2011; Cheng et al. 2012; Daquinag et al. 2013; Kapur and Katz 2013; Kyurkchiev et al. 2014; Madrigal et al. 2014; Dubey et al. 2018; Ferreira et al. 2018; L et al. 2019; Lombardi et al. 2019; Meiliana et al. 2019). The MSCs secretome in general consists of such biologically active factors as chemokines and cytokines, cell adhesion molecules, lipid mediators, interleukins (ILs), growth factors (GFs), hormones, micro RNAs (miRNAs), long non-coding RNAs (lncRNAs), messenger RNAs (mRNAs), exosomes, as well as microvesicles (Kyurkchiev et al. 2014; Madrigal et al. 2014; Dubey et al. 2018; Ferreira et al. 2018; Lombardi et al. 2019; Meiliana et al. 2019). It is revealed that MSC secretion include in particular vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF-b1), nerve growth factor (NGF), placental growth factor (PGF), stromal-derived growth factor (SDF-1/CXCL12), monocyte chemo-attractant protein-1 (MCP-1/CCL2), IL-6, IL-8, IL-10 and IL-13 (Meiliana et al. 2019), bone morphogenetic proteins (BMP), CC chemokine ligand 5/Regulated on activation, normal T cell expressed and secreted (CCL5/RANTES), epidermal growth factor (EGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), inter-cellular adhesion molecules (ICAM), indoleamine-2,3-dioxygenase (IDO), leukemia inhibitory factor (LIF), matrix metalloproteases (MMP-1, MMP-2, MMP-3, MMP-7), platelet-derived growth factor (PDGF), metalloproteinase inhibitors (TIMP-1, TIMP-2) (Polacek et al. 2011; Osugi et al. 2012; Inukai et al. 2013; Kyurkchiev et al. 2014; Pereira et al. 2014; Ferreira et al. 2018; Linero and Chaparro 2014).

Herewith, mesenchymal stem cells of various origin, including bone-marrow-derived mesenchymal stem cells (BM-MSCs), adipose tissue-derived stem cells (ADSCs) or human uterine cervical stromal stem cells (hUCESCs), may be good candidates for obtaining products from secretome (Zhao et al. 2013; Vizoso et al. 2017). However different populations of MSCs should be used for specific purposes because the composition of the secretome depends on the stromal cells source. For instance, ADSCs have higher expression of mRNA, VEGF-D, IGF-1 and IL-8, while dermal-sheath- and dermal-papilla-derived cells secrete higher concentrations of CCL2 and leptin (Hsiao et al. 2012). It is known that placenta-derived MSCs are characterized with increased expression levels of HGF, bFGF, IL-6, IL-8, IL-1a and IL-1b, while in secretome obtained from bone marrow-derived MSCs the levels of VEGF-A, NGF and angiogenin are higher (Du et al. 2016; Meiliana et al. 2019).

There is evidence that the molecules produced by MSCs (secretomes), especially those packaged in extracellular vesicles (EVs), influence the tissue repair even better than the cells themselves (Lepperdinger et al. 2008; Madrigal et al. 2014; Dubey et al. 2018; Ferreira and Gomes 2018; Pelizzo et al. 2018; Campanella et al. 2019; Lombardi et al. 2019; Mitchell et al. 2019).

Therefore due to the paracrine activity, MSCs conditioned medium (CM) or purified MSC-derived extracellular vesicles having a plethora of bioactive factors are assumed to have the most prominent cell-free therapeutic impact and can serve as a better option in the field of regenerative medicine in the future (Maguire 2013; Zhou et al. 2013; Vizoso et al. 2017; Park et al. 2018; L et al. 2019; Lombardi et al. 2019).

This new frontier of research provides several key advantages over cell based applications: (a) the administration of proteins instead of whole cells as a new therapeutic option in regenerative medicine; (b) CM can be stored without any toxic cryopreservatives, such as dimethyl sulfoxide (DMSO), for a relatively long period; (c) preparation of CM is more economical as it can be mass-produced from the available MSC populations under current good manufacturing practice (cGMP) conditions; (d) evaluation of CM for safety and efficacy will be much simpler and analogous to conventional pharmaceutical agents (Bermudez et al. 2015; L et al. 2019).

Anti-inflammatory activity

It is well known that there are anti-inflammatory factors in the MSCs secretome, including tumor necrosis factor β1 (TGFβ1) (Zagoura et al. 2012), interleukin 13 (IL13) (Bermudez et al. 2016), interleukin 18 binding protein (IL18BP), ciliary neurotrophic factor (CNTF), neurotrophin 3 (NT-3) factor, interleukin 10 (IL10), interleukin 12 p70 (IL12p70), interleukin-25 (IL-25), which is also known as interleukin-17E (IL17E), interleukin 27 (IL27), or interleukin 1 receptor antagonist (IL1RA). On the other hand, pro-inflammatory cytokines are also present in MSCs conditioned medium, for example, IL1b, interleukin 6 (IL6) (See et al. 2011; Cantinieaux et al. 2013), interleukin 8 (IL8) (Mirabella et al. 2011), and interleukin 9 (IL9) (Lee et al. 2011). Thus, the balance between the anti- and pro-inflammatory factors will determine the final effect of conditioned medium on the inflammatory process.

However, the numerous studies highlight MSCs anti-inflammatory effect. Yi and Song (2012) described that MSCs inhibited proinflammatory cytokines, such as interferon (IFN)-γ and tumour necrosis factor α (TNFα), while increasing release of anti-inflammatory IL10. Legaki et al. (2016) showed that hUCESC-CM treatment significantly reduced mRNA expression of pro-inflammatory cytokines (IL6, IL8, TNFα and macrophage inflammatory protein-1 alpha (MIP-1α)), but increased mRNA expression of the anti-inflammatory cytokine (IL10). The similar results were obtained during experiments with MSC-CM from

amniotic fluid in a mice colitis model (Legaki et al. 2016). It was also found that hUCESC-CM reduced the infiltration of leucocytes in ocular tissues (Vishnubhatla et al. 2014).

Moreover, anti-inflammatory cytokines in MSC-CM can contribute to the beneficial effects seen in animal models of diabetes, acute colitis, inflammatory arthritis, etc. (Brini et al. 2017; Kay et al. 2017; Pouya et al. 2018). It was revealed that single intravenous injection of a conditioned medium derived from adipose tissue (hAT-CM) into streptozotocin-(STZ)-treated diabetic mice relieved the diabetic neuropathic pain by re-establishing the Th1/Th2 balance with a long-lasting relief of sensory hypersensitivity. In the experiments with the STZ-treated diabetic mice, it was demonstrated that the content of anti-inflammatory and immunomodulatory cytokines (IL-1β, IL-6 and TNF-α) in dorsal root ganglia, sciatic nerves and spinal cord restored to basal levels after 1 week of hAT-CM injection. The elevated level of IL-10 also confirmed realization of an anti-inflammatory mechanism (Brini et al. 2017). Pouya et al (2018) showed that an intraperitoneal injection of MSC-CM in C57BL/6 mice with colitis mediated a significant decrease in colon inflammation and an increase in colon weight and length, which led to the disease activity index and mortality rate reducing. Furthermore, the mesenteric lymph nodes and spleen of the mice infused with MSC-CM demonstrated increased levels of the anti-inflammatory cytokines IL-10 and TGF-β and reduced levels of the pro-inflammatory cytokine IL-17 confirming the anti-inflammatory role of CM. Similarly, in the antigen-induced model of inflammatory arthritis, it was shown that an intra-articular injection of murine MSC-CM was effective in reducing disease severity and cartilage damage. The high levels of IL10 in CM were revealed, which correlated with an anti-inflammatory response (Kay et al. 2017; L et al. 2019).

In order to study whether the anti-inflammatory potential of ADSC secretome is higher than EV-enriched fraction of ADSCs secretome, the effect of both fractions was investigated on the TNF-α-induced nuclear translocation of the NF-κB subunit p65 in U251 cells. It is interesting to note that the level of nuclear NF-κB p65 was significantly increased by TNF-α treatment compared to control cells. The effect of the total secretome fraction on TNF-α stimulated cells was accompanied by a non-significant reduction of nuclear p65, whereas the influence of EV fraction led to a significantly reduced amount of p65 (Mitchell et al. 2019).

Anti-apoptotic activity

There are studies which illustrate that MSCs produce inhibitor proteins of apoptosis to restore local environment and prevent therefore cell death (Li et al. 2015). Tang et al. (2005) reported that MSCs decreased the pro-apoptotic factors expression (Bax and cleaved caspase-3) and stimulated at the same time the anti-apoptotic compounds levels (Bcl-2). It is noteworthy that MSCs treatment of hearts led to elevated level of pro-angiogenic factors expression, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and stromal cell-derived factor-1 (SDF-1), which is also called chemokine (C-X-C motif) ligand 12 (CXCL12) (Tang et al. 2005).

There are also studies revealing a pro-apoptotic effect of hUCESC-CM on malignant cells. In accordance with these data, the effects of MSCs on normal and cancer cells are different. Along with an antiapoptotic effect of hUCESC-CM on normal cells (Bermudez et al. 2016), the apoptosis occurred in cancer cells under the influence of conditioned medium obtained from human uterine cervical stem cells *in vitro* and *in vivo* (Eiró et al. 2014; Vizoso et al. 2017).

Anti-fibrotic activity

An anti-fibrotic effect of stem cells conditioned medium is mediated by bioactive molecules in MSCs secretome which decrease accumulation of extracellular proteins and, therefore, lead to reduced scar formation. An et al. (2017) studied the influence of umbilical cord-derived mesenchymal cells (UCMSC) secretome on formation of fibrotic areas in mice with hepatic fibrosis. A decrease in the number of activated hepatic stellate cells (HSCs) expressing α- smooth muscle actin (α-SMA) was shown after an injection of the UCMSC-CM in the diseased mice, which was accompanied by reducing fibrotic areas. The researchers analysed the UCMSCs secretome using nano-chip-LC/QTOF-MS and discovered the presence of milk fat globule EGF factor 8 (MFGE8), an anti-fibrotic protein known to down-regulate the expression of TGF-βR1 (transforming growth factor β type 1 receptor) at the mRNA and protein level, thereby decreasing the activation of human hepatic stellate cells (An et al. 2017).

Paracrine effect of MSCs in bone regeneration

It was revealed that secretome synthesized by different stem cells, including rat bone marrow-derived MSCs (rBM-MSCs), human adult mesenchymal stem cells (haMSCs) and human fetal mesenchymal stem cells (hfMSCs), promoted osteogenic differentiation of rBM-MSCs. It is quite important that the human MSC conditioned medium effects in the same way as rat MSCs secretome, or even better. Xu et al. (2016) described that the hfMSCs secretome is characterised as a conditioned medium with the strongest osteogenic induction ability compared to the conditioned medium obtained when cultivating rBM-MSC or haMSC. It was also revealed that hfMSC conditioned medium does not induce any significant immune response, which makes it different from the haMSC secretome. At the same time, conditioned media at different concentrations did not affect rBM-MSC viability or cell proliferation. Furthermore, hfMSCs secretome at the concentration of 100 μg/μl could enhance mineralization during rBM-MSC osteogenic induction

via increasing of alkaline phosphatase (ALP) activity and formation of calcium nodules.

The expression levels of osteogenic marker genes, including runt-related transcription factor 2 (Runx2), osteocalcin (OCN), osteopontin (OPN), and osterix (Osx), were significantly upregulated on Days 3 and 10 after the hfMSCs secretome treatment. ALP is responsible for osteoblastic differentiation at an early stage; it hydrolyses pyrophosphyte and generates inorganic phosphate which promotes the process of mineralization. Runx2 produces bone matrix proteins and is essential for osteoblast differentiation. Growth and differentiation factors regulate expression levels of OPN and mediate bone formation and its remodeling. Osx acts downstream of Runx2, this factor takes part in the processes of osteoblast differentiation and bone tissue formation. It was shown that conditioned medium obtained from hfMSC stimulated differentiation of rBM-MSCs in osteogenic direction *in vitro*. Furthermore, application of the hfMSCs secretome locally into distraction osteogenesis gap in rats led to accelerated new bone formation and consolidation (Xu et al. 2016).

It is known that during the distraction osteogenesis procedure proliferation of bone progenitor cells increases and their recruitment to the target site occurs. The healing process is accompanied by the angiogenesis and bone tissue formation/mineralization. At the same time, the mechanisms of the hfMSCs conditioned media effect on bone tissue regeneration, including vascular network formation and remodelling, remain unknown. There is evidence that the MSCs secretome mediates the release of vascular endothelial growth factor (VEGF) at the site of tissue repair, being stimulated by hypoxia or normoxia. It is known that the expression of VEGF enhances bone tissue and blood vessels formation during distraction osteogenesis, and this biological active factor is required for osteogenic differentiation.

Moreover, osteogenic lineage commitment of MSCs is accompanied by the Osx and OCN osteoprogenitors markers expression. Treatment of the damaged area with the hfMSCs secretome upregulates the number of Osx- and OCN-positive osteoprogenitors in the distraction zone in comparison with the control group. Different signaling pathways may be involved in VEGF production following hfMSC conditioned medium influence, and further experiments are required to make the molecular mechanisms of these processes clear (Xu et al. 2016).

Neuroprotective effect and impact on neural/glial proliferation

There have been several studies in which adult stem cells, including mesenchymal stem cells, were used as a possible tool for central nervous system (CNS) regeneration (Lindvall and Kokaia 2010; Shihabuddin and Aubert 2010; Kan et al. 2011; Teixeira et al. 2014), which could be a promising therapeutic option (Pittenger et al. 1999; Zuk et al. 2002; Wang et al. 2004; Teixeira et al. 2014).

It was shown that the MSCs secretome mediated neuroprotective and neurotrophic effects (Caseiro et al. 2016; Luarte et al. 2016; Ratajczak et al. 2016) due to a number of neurotrophic factors (de Almeida et al. 2014; Mead et al. 2014). There are studies which demonstrate on nerve injury models that the MSC-based approach generated healing effects, including enhanced vascularization of the regenerating site, increased thickness of the myelin sheaths, modulation of the Wallerian degeneration stage, accelerated fibre regeneration, reduction of fibrotic scaring, and improved fibre organization (Caseiro et al. 2016).

It was discovered on experimental animal models that stem cells obtained from bone marrow (BM-MSCs) and adipose tissue (ASCs) improved the healing process after stroke (Wei et al. 2009; Honmou et al. 2012; Teixeira et al. 2014), demyelination (Constantin et al. 2009; Cristofanilli et al. 2011; Teixeira et al. 2014), Parkinson's disease (Cova et al. 2010; Erba et al. 2010), and spinal cord injury (Arboleda et al. 2011; Park et al. 2012). To restore the central nervous system (CNS), the stem/progenitor cells from different sources could be used. For example, stem cells present in the Warton jelly of the umbilical cord, known as Wharton jelly stem cells (WJ-MSCs) and human umbilical cord perivascular cells (HUCPVCs), have a great potential in healing CNS injuries (Salgado et al. 2010; Datta et al. 2011; Taghizadeh et al. 2011). Populations of WJ-MSCs and HUCPVCs are also identified as mesenchymal stem cells (Sarugaser et al. 2005; Weiss and Troyer 2006; Baksh et al. 2007; Sarugaser et al. 2009). The major effects of MSCs are supposed to be determined by their secretomes (Salgado et al. 2010; Carvalho et al. 2011; Ribeiro et al. 2012; Teixeira et al. 2013; Teixeira et al. 2014). Both neural stem cells (NSCs) and MSCs secrete a variety of growth factors (Salgado et al. 2015). It was shown that the molecular content of the MSCs secretome depended on the culture duration and tissue sources of MSCs, and it influenced significantly the changes of primary cultures of hippocampal neurons and glial cells viability (Kim et al. 2013).

During the *in vitro* experiments, the ability of the HUCPVCs conditioned media to modulate the survival and viability of both neuronal and glial cells populations was shown (Salgado et al. 2015). It was demonstrated that application of HUCPVCs-CM to human telencephalon neural precursor cells (htNPCs) *in vitro* led to an increase of neuronal cell differentiation, which was characterised by higher densities of immature (DCX+ cells) and mature neurons (MAP-2+ cells). Moreover, an injection of HUCPVCs and their secretome into the dentate gyrus (DG) was accompanied by increasing the endogenous proliferation (BrdU+ cells) in a week. It was revealed that application of HUCPVCs led to an increased number of newborn neurons (DCX+ cells). And an injection of CM or HUCPVCs into the DG tissue promoted an elevated level of fibroblast growth factor-2 (FGF-2) and, to a lesser extent, of nerve growth factor (NGF). Thus, either transplantation of HUCPVCs or the application of their conditioned media potentiated enhanced neuronal viability and

differentiation *in vitro* and *in vivo* (Teixeira et al. 2014; Salgado et al. 2015).

The effect of intranasal application of CM derived from stem cells of human exfoliated deciduous teeth (SHEDs) in an animal model of Alzheimer's disease was studied. And the cell-free treatment was accompanied by an improvement of cognitive function and induced neuroregenerative effects, for example, an attenuated pro-inflammatory response induced by amyloid plaques, and anti-inflammatory M2-like microglia (Mita et al. 2015; Vizoso et al. 2017).

It was revealed that application of a secretome obtained from hypoxic-preconditioned MSCs promoted reduce of neuronal cell loss and apoptosis and production of VEGF which stimulated recovery processes in the organisms of traumatic brain injury-induced rats (Chang et al. 2013).

Angiogenesis and revascularization

Angiogenesis is a process of new vasculature sprouts formation from pre-existing blood vessels. This process normally occurs during wound healing. Numerous studies illustrated a particular impact of the MSCs secretome at the different stages of angiogenesis (Burlacu et al. 2013).

The effect of MSCs on the process of angiogenesis is studied in different spectrum of diseases, including impaired vessel growth during atherosclerosis and wound healing. Several studies demonstrated that the MSCs application led to stimulation of blood vessels formation in animal models of myocardial infarction, neurogenic bladder, peripheral artery disease, stress urinary incontinence, and cerebral ischemia/stroke (Hsieh et al. 2013; Liu et al. 2013; Sharma et al. 2013).

The MSCs secretome contains numerous biologically active molecules which act as angiogenic stimulators and inhibitors (Kinnaird et al. 2004; Di Santo et al. 2009; Boomsma et al. 2012; Ho et al. 2012). An extensive proteomic analysis of the conditioned media of mesenchymal stem cells stimulated with inflammatory cytokines revealed the presence in a secretome of tissue inhibitor of metalloproteinase-1 (TIMP-1) which is responsible for the MSCs antiangiogenic impacts (Zanotti et al. 2016). Moreover, some studies showed the dependence of the pro- and anti-angiogenic factors secretion on chemokines and hypoxic conditions. In particular, to describe the effect of bioactive molecules on MSCs secretion ability, it is important to note that TGFα increases the level of growth factors in the secretome (i.e., VEGF, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), IL6 and IL8). And a conditioned medium from MSCs treated with TFG-α induces blood vessel growth in an *in vivo* assay (De Luca et al. 2011; Vizoso et al. 2017). The obtained data demonstrate the complicated set of bioactive molecules in the MSCs secretome which can be balanced under the different interventions to promote angiogenesis.

The ASCs secretome also has a proangiogenic effect, which was demonstrated in acute myocardial infarction models. Rehman et al. (2004) showed that the conditioned media obtained from ASCs stimulated angiogenesis in the ischemic environment. The following bioactive molecules were detected in the secretome after the analysis: G-CSF, TGF-β, VEGF, HGF, and bFGF (Rehman et al. 2004; Kapur and Katz 2013). The impact of HGF on the formation of blood vessels was proved through restricting the production of this factor by ASCs, which resulted in a decreasing effect by ASCs on endothelial cell proliferation, migration and survival in the ischemic environment (Cai et al. 2007).

Cutaneous wound healing

A cutaneous wound healing is a fascinating process, which requires cell migration, proliferation, matrix protein synthesis, and tissue remodelling. In particular, keratinocytes are involved in the epithelialization and dermal repair, and endothelial cells promote angiogenesis (Kober et al. 2016). The migration and proliferation of fibroblasts are the key processes in a wound healing mechanism. In the early stage of wound repair, they move to the damaged region and promote blood vessels regeneration and granulation tissue formation. In the advanced trauma stage, fibroblasts mature into myofibroblasts which are responsible for wound closure process (Zhao et al. 2013).

It was revealed that the adipose stem cell-conditioned medium (ASC-CM) had a marked stimulating effect on cutaneous wound healing, via affecting the mechanism for this response by influencing effector cells (Lombardi et al. 2019).

An increased proliferation and migration activity of primary human dermal fibroblasts (HDFs) as well as type I collagen secretion was shown after ASCs secretome application (Kim et al. 2007). Kober et al. (2016) revealed a stimulatory effect of ASC-CM on ASCs, and the proliferation of keratinocytes after application of ASCs secretome was significantly reduced. Furthermore, addition of ASC-CM did not affect cell migration, which had been tested with *in vitro* scratch assays. Collawn et al. (2016) demonstrated on a 3D skin model that application of ASC-CM as well as ASCs promoted an acceleration of wound repair. Seo et al. (2017) showed that ASC-CM, similarly to ASCs, stimulated the proliferation, migration, and invasion properties of HUVECs. An addition of ASC-CM led to increasing HaCaT cell proliferation and migration, as well as vascular endothelial growth factor (VEGF) secretion. Cooper et al. (2018) demonstrated that application of ASC-CM stimulated HDF migration ability *in vitro*. Park et al. (2018) collected CM samples from cultured ASCs isolated from adipose tissues of breast cancer patients and added the secretome to HDFs, normal adult human primary epidermal keratinocytes (HEKa), or HUVECs cultures. It resulted in increased cell proliferation, migration, and invasion. Kim et al. (2018) showed that ASC-CM from 3D-cultures influenced more significantly on the proliferation of HaCaT cells than the one obtained from 2D-cultivated ASCs. Noverina et al. (2019) analyzed the growth factor profile in ASC-CM via immunosorbent assay (ELISA) method and revealed a higher concentration level of fibroblast growth

factor (FGF), which is involved in wound healing and regeneration. Stojanovic and Najman (2019) demonstrated the immunomodulatory and wound healing potential of the secretome collected from stem cells previously isolated from adipose tissue, or lipoma (Lombardi et al. 2019).

Some studies described the presence of growth factors in the MSCs secretome that promoted tissue regeneration with a special focus on proliferation (Lee et al. 2011; Zagoura et al. 2012; Turner et al. 2013; Bhang et al. 2014; Bermudez et al. 2015). The experimental myocardial infarction models showed that anti-fibrotic and angiogenic effects of the MSCs secretome inhibited scar formation process (Cargonini et al. 2012; Preda et al. 2014) and stimulated the synthesis and consequent secretion of bioactive molecules responsible for remodelling (Williams et al. 2013; Vizoso et al. 2017).

It is known that such components of the ASCs secretome as bFGF significantly stimulate migration and proliferation of functional cells in wound healing; however PDGF-AA and VEGF influence only fibroblasts migration (Zhao et al. 2013). It was revealed that EGF could accelerate reepithelialisation process via stimulating the keratinocytes proliferation and migration in an acute wound. Zhao et al. (2013) demonstrated an increased proliferation of fibroblasts under the EGF influence. However, the mechanism of the EGF effect on cells migration is still unclear.

Hu et al. (2013) showed that wound healing was mediated via application of the ASCs secretome which stimulated migration of vascular endothelial cells 4 hours later, fibroblasts 12 hours later and then keratinocytes 24 hours later.

Park et al. (2018) reported that elevated levels of EGF, bFGF and HGF in conditioned media promoted wound healing.

And finally, it was revealed that increasing proliferative and migratory characteristics of different dermis cellular components, including dermal fibroblasts, keratinocytes, and endothelial cells, *in vitro* occurred by activating PI3K/Akt and FAK-ERK1/2 signaling (Park et al. 2017).

Muscle regeneration

Tissue regeneration and homeostasis are mediated by cell proliferation, migration and stem cell differentiation processes. Mitchell et al. (2019) showed that application of the total secretome mouse myoblast cell line C2C12 for 48 hours resulted in increased cell proliferation in comparison with the control group. Moreover, the total secretome stimulated the differentiation of C2C12 cells into myofibers (Mitchell et al. 2019).

Mitchell et al. (2019) studied the effect of the total ASCs secretome and its EV fraction on tissue repair in a mouse model of acute cardiotoxin-induced muscle injury. It was revealed that the total ASCs secretome stimulated the process of tissue regeneration, which was confirmed by a significant decrease in the activity of lysosomes in the group of animals treated with the total ASC-CM (Mitchell et al. 2019). Studying the cross-sectional area of newly formed muscle fibres during the regeneration process revealed that the application of EV fraction had a greater effect than the total ASCs secretome (Mitchell et al. 2019).

Liver regeneration

Lee et al. (2014) demonstrated that liver regeneration in partially hepatectomised models occurred after systemic infusion of the ASCs secretome. It was shown that application of ASCs-CM increased mRNA expression of Lgr5 (a Wnt target gene), which was an indicator of actively dividing stem cells. Expression of Lgr5 occurred in small cells located near bile ducts as a result of a liver cell injury (Huch et al. 2013; Lee et al. 2014). During the repair phase, those cells were able to generate significant numbers of hepatocytes and biliary duct cells and, thus, could be considered a class of liver progenitor cells. Higher expression of Lgr5 in small cells located near bile ducts is an indicator of liver regeneration (Huch et al. 2013; Lee et al. 2014).

Lee et al. (2014) showed an increased expression of p-Akt, p-Erk1/2, which are the downstream effector components of HGF signaling pathways, and p-STAT3, which is responsible for cell cycle progression from G1 to S phase, after application of the ASC-secretome (Lee et al. 2014), which indicated that the ASC-secretome promoted liver regeneration.

Conclusion

The MSC-conditioned medium, or secretome, contains a plethora of cytokines and a wide array of bioactive factors, such as chemokines, cell adhesion molecules, lipid mediators, IL, growth factors, hormones, exosomes, microvesicles, etc., which are secreted by MSCs (Lee et al. 2019). These factors have been considered as protagonists to participate in tissue repair and regeneration through their paracrine actions that mediate cell-to-cell signalling (Madrigal et al. 2014).

It is critical for the success of such a cell-free therapy to identify, analyze and elucidate the mechanism of action of each component of the secretome.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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