Secretion, migration and adhesion as key processes in the therapeutic activity of mesenchymal stem cells*

Marta Kot, Aleksandra Musiał-Wysocka, Małgorzata Lasota, Aleksandra Ulman and Marcin Majka

Jagiellonian University, Medical College, Department of Transplantation, Kraków, Poland

The MSCs are immature cells that can be found in numerous different tissue types. In recent years, they have gained considerable attention, particularly with regard to their regenerative properties. Due to their paracrine activity, ability to migrate, adhesion and homing, MSCs currently appear to be the most relevant for therapeutic use. Numerous bioactive molecules secreted by MSCs exert paracrine effects and modulate many physiological processes, such as angiogenesis, immunomodulation and neuroprotection. Cell-cell communication may be also mediated by extracellular vesicles released from the cells. Due to these properties, MSCs have been widely studied for evaluation of their therapeutic benefits expected in the clinical applications. For effective tissue regeneration, transplanted MSCs have to exit the circulation and locate at the site of damage, which is possible because of their ability to migrate, adhere and engraft at the target site. Accumulating evidence suggests that MSCs recruitment from remote sites is similar to leukocytes’ migration. All of these biological features make MSCs highly investigated stem cells and the most commonly used cells in regenerative medicine. Since environmental factors affect the MSCs behavior, we discuss importance of oxygen concentration as one of the key factors affecting MSCs properties.

Key words: mesenchymal stem cells (MSCs), secretion, adhesion, migration, homing, cell therapy

Received: 21 October, 2019; revised: 17 December, 2019; accepted: 19 December, 2019; available on-line: 28 December, 2019

✉ e-mail: mmajka@cm-uj.krakow.pl (MM); marta.kot@uj.edu.pl (MK)
*Acknowledgements of Financial Support: The costs of the article published as a part of the 44th FEBS Congress Kraków 2019 – From molecules to living systems block are financed by the Ministry of Science and Higher Education of the Republic of Poland (Contract 805/P-DUN/2019). This work was supported by a research grant (STRATEGMED2/265761/10/NCBR/2015) from the National Center for Research and Development.

Abbreviations: MSCs, mesenchymal stem cells; EVs, extracellular vesicles; SDF-1α, cell-derived factor-1 alpha chemokine; MMP1, matrix metalloproteinase-1; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor 1 alpha

INTRODUCTION

Mesenchymal stem cells (MSCs) are self-renewing, multipotent cells that can be isolated from nearly every tissue type (bone marrow, adipose tissue, umbilical cord, peripheral blood, placenta) (Zvaifler et al., 2000; Zuk et al., 2001; Najar et al., 2010; Fei et al., 2013; Heidari et al., 2013; Meier et al., 2013; Musialek et al., 2015; Musiał-Wysocka et al., 2019a,Musiał-Wysocka et al., 2019b). A wide variety of tissue sources implies high heterogeneity of the isolated populations. Thus, MSCs are identified according to criteria established by ISTC in 2006, which have been summarized by Dominici and others (Dominici et al., 2006). An important property of MSCs is their ability to secrete numerous factors involved in different processes that favor tissue remodeling, such as angiogenesis, immunomodulation or neuroprotection. All of these biological features, combined with ease of culture expansion, make MSCs highly investigated stem cells and the most commonly used cells in regenerative medicine (Lee et al., 2010; Wang et al., 2012; Wei et al., 2013; Hare et al., 2017). It should be noted that despite general unified criteria outlined for MSCs, the cells isolated from different tissues may vary in terms of their potential to differentiate, proliferate or their profile of secreted factors. MSCs retain properties of the microenvironment from which they are derived in vivo (Mrozik et al., 2010).

The available data allow to distinguish some mechanisms that are responsible for the therapeutic potential of mesenchymal stem cells. Positive effect of exogenously injected MSCs can be a result of both, the cell-cell interaction and the paracrine activity, such as:
• the ability to secrete factors initiating healing and tissue regeneration
• horizontal material transfer (microvesicles, exosomes, mitochondria)
• the ability to reduce inflammation and regulate the immune response
• the capacity to differentiate/transdifferentiate into various cell lineages
• the ability to migrate and home in the site of injury
• fusion with the surrounding cells

One of the most important challenges of the current clinical use of MSCs is the comprehensive understanding of their biology, in particular the mechanism underlying the basis of their ability to regenerate damaged tissues. Despite accumulating evidence, the nature and functions of MSCs are not fully understood. Taking into consideration all MSCs properties known so far, it is difficult to indicate only one of them that might be responsible for the therapeutic abilities of these cells. In this paper, we discuss the current understanding of MSCs properties that are inseparably connected with their regenerative potential, such as the secretory activity and ability to migrate, adhere and home. It should be noted that the in vitro culture conditions under which the cells are examined differ from the in vivo environment, thus we have also considered the importance of oxygen concentration in MSCs investigations.
THE MSCS’ PARACRINE EFFECT: SECRETOME AND MSCS-RELEASED VESICLES

MSCs secretome

The ability to secrete a variety of bioactive factors (cytokines, chemokines, and growth factors) is one of the key activities of MSCs. The released molecules are involved in interactions between MSCs or the surrounding microenvironment.

The spectrum of compounds produced and released by MSCs, generally referred to as the MSCs secretome, can be divided into several panels of factors involved in various biological processes, i.e. angiogenesis, immunomodulation, neuroprotection, proliferation, migration, and chemotaxis (Fig. 1).

MSCs are tested in many preclinical and clinical studies as a promising approach for various therapies. They gained special attention in tissue regeneration, where the paracrine effect of MSCs is of particular interest. The ability to secrete biologically active molecules that can affect nearby cells is considered as key MSCs activity. It depends on the histological source of cells, the donor age and impact of the surrounding microenvironment. MSCs secretome composition can vary significantly (Doorn et al., 2012). It is abundant in growth factors, cytokines and chemokines, therefore making MSCs a perfect source of a conditioned media for many studies concerning cell-free therapy (Praveen et al., 2019). Based on the composition and biological effects, we can divide the MSCs secretome into immunomodulating, neuroprotective, tissue remodeling and angiogenic.

Immunomodulation

MSCs’ properties of regulating the immune system are based not only on the particular surface molecules but also on secretion of many soluble factors. MSCs secrete both, the anti-inflammatory cytokines such as the transforming growth factor β1 (TGF-β1), interleukin 13 (IL-13), and pro-inflammatory cytokines such as IL1β, IL6, IL8 and IL9 (Vizoso et al., 2017). The importance of this equilibrium is presented with the results from a study with a mouse model of diabetes. It was shown that after application of conditioned media from the adipose tissue, the MSCs balance in the pro- and anti-inflammatory factors was restored. The IL-10 level was decreased, whereas the IL-18, IL-6 and TNF-α levels were elevated in the dorsal root ganglia and the spinal cord (Brini et al., 2017).

Angiogenic

MSCs are widely studied in research concerning ischemic recovery. Angiogenic properties that promote sprouting of new blood vessels from preexisting ones seem to be a crucial regenerative feature of MSCs. Many studies confirm this activity owing to production of a variety of factors, such as the vascular endothelial growth factor (VEGF-A), angiopoietins (ANGPTs), insulin-like growth factor-1 (IGF-1) or hepatocyte growth factor (HGF). VEGF is indicated as one of the most abundant angiogenic secretome properties. It was shown by Oskowitz et al. (2011) that a serum deprivation condition results in an increased expression of angiogenic factors, such as the VEGF-A, ANGPTs, IGF-1, and HGF. The angiogenic potential of the secretome was confirmed using an ex vivo rat aortic ring assay where media from serum deprived MSCs resulted in significantly longer vascular sprouts from rat aortic rings, in comparison to the unconditioned media. Apparently, the serum level is more relevant than the oxygen level – minor differences were observed in angiogenic properties in conditioned media from MSCs obtained under normal and hypoxic conditions (Burlacu et al., 2013).

Tissue-remodeling and anti-fibrotic activity

Scar formation is an inseparable process of wound healing. MSCs have an ability to reduce accumulation of the extracellular matrix proteins. In the secretome from umbilical cord mesenchymal stem cells (UC-MSCs) in mice with fibrosis, 32 proteins were identified which downregulate expression of fibrotic factors, such as the metalloproteinases, collagens, TGF-β and Smad proteins. The milk fat globule EGF factor 8 (MFGE8) negatively regulates expression of TGFβR1 transforming growth factor β type 1 receptor, and therefore acts as an anti-fibrotic factor (An et al., 2017). The hypoxic environment promotes expression of cytoprotective factors in Akt1 overexpressing BM-MSCs (Gneechi et al., 2005).

Neuroprotection

The nervous system is characterized by a low regeneration potential. Therefore, studies that associate MSCs with improved nervous system recovery are substantial for the future therapies. In the beginning those functions were correlated with the differentiation capacity of MSCs, but recent studies reveal the importance of the MSCs secretome in which we can find many neuroprotective and neuroregulatory factors (Teixeira et al., 2013). Crigler and others (Crigler et al., 2006) discovered transcripts coding a brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), after analyzing MSCs cDNA library. What is more, they correlate the nerve survival and outgrowth improvement with BDNF activity from co-culture experiments (Crigler et al., 2006). Further studies reveal that BDNF is a key factor secreted by MSCs from Wharton’s Jelly of the umbilical cord (WJ-MSCs), which exclusively mediates axonal elongation in the rat cortical and hippocampal neurons (Martins et al., 2017).

MSCs-RELEASED VESICLES – HORIZONTAL MATERIAL TRANSFER AND CELL-TO-CELL COMMUNICATION

Extracellular vesicles (micro vesicles (MVs) and exosomes) are small membrane vesicles (30–150 nm in
diameter) of endocytic origin (Simpson et al., 2008). They arise from intracellular membrane of the cell by inward budding and are released from the cells into extracellular space after fusion with the plasma membrane (Mears et al., 2004). During their formation, various transmembrane proteins and cytosolic components are incorporated into membrane invagination and closed within the vesicles (van Niel et al., 2006). It has been found that MSCs can produce higher quantities of exosomes than other cells (Yeo et al., 2013). Extensive composition analysis of EVs released from MSCs has revealed occurrence of over 900 proteins. Examples of some identified proteins are depicted in Table 1. Among the set of cellular proteins, EVs may also contain a variety of lipids and different kinds of RNAs (mRNAs, microRNA, tRNA, long noncoding RNAs) (http://exocarta.ludwig.edu.au). In general, the exosomes comprise tissue- or cell-type specific molecule composition depending on the tissue type in which they arise. In MSCs-derived EVs, CD73, CD90 and CD105 antigens have been found that are characteristic markers for MSCs (Dominici et al., 2006).

Recently, cell-cell communication mediated by extracellular vesicles has gained scientific interest. It is believed that a possible mechanism of intercellular communication through exosomes follows three pathways (Threy et al., 2001; Janowska-Wieczorek et al., 2005; Bi et al., 2007; Camussi et al., 2010; Raposo & Stahl, 2019):

- juxtaacinar signaling (contact-dependent signaling) – proteins occurring in the exosomal membrane can interact with receptors in the membrane of target cells which activates intracellular signaling;
- ectodomain cleavage-based signaling – the exosomal membrane proteins are cleaved by proteases, released into extracellular space and then they become ligands which bind to the cell surface receptors;
- fusion – exosomes fuse with the cell membrane and release their contents into the recipient cell cytoplasm.

Several studies have reported that MSCs-derived exosomes perform functions similar to those of MSCs, including repairing tissue and suppressing the inflammatory response. For this reason, MSCs-derived extracellular vesicles seem to be an alternative to MSCs in medical applications. The cell-free therapy offers safety and low immunogenicity with the same regenerative capacities as the mother cells (Ragert et al., 2016; Willis et al., 2018). The use of MSC-conditioned media without cell transplantation can promote tissue repair, which supports a potential role of EVs in regenerative medicine (Chen et al., 2014; Tsai et al., 2014).

### Table 1. Proteins identified in MSCs derived exosomes (data from http://exocarta.org).

| Protein name | Function |
|--------------|----------|
| GAPDH, enolase 1, aldolase 1, PKM2, PGK1, PDA3, GSTP1, DPP4, AHCY, TPL1, peroxiredoxins, P4HB, LDH, cyclolinophin A, FASN, MDH1 and CNP | Metabolic enzymes |
| MFGE8 and integrins | Adhesion |
| HSP60, HSP70, HSPA5, CCT2 and HSP90 | Heat-shock proteins |
| Annexins: I, II, IV, VI, VII and X | Membrane trafficking and fusion |
| Syntenin, 14-3-3, G proteins, ARF1, CDC42, stomatin, SLC9A3R1, RALA, PDCD6, rac1, mcin 1, EHD1, RAN, PEBP1, MIF, RRS2, RAC1, NRAS and EHD4 | Signal transduction |
| Vimentin, actins, tubulins, coflin 1, ezrin, profilin 1, moesin, radixin, myosin, perlecan, THBS1, IQGAP1, keratins, gelsolin, fibronectin 1 and LGALS3BP | Cytoskeletal proteins |

### FUSION – DIRECT CELL-CELL INTERACTION

One of the possible mechanisms of MSCs action may be fusion with the host cells. Cell fusion is an omnipresent process in life and there is also evidence for fusion processes between stem cells and other neighboring cells. The mechanism of stem cells fusion with other tissue cells remains elusive as potentially therapeutic. The discussion is still ongoing, partially because of the frequency of fusion events that are very rare. This is probably the main reason why we can find only a few papers describing the fusion process of MSCs. Several studies have reported that stem cells can fuse with cardiomyocytes either by a permanent or partial cell fusion process (Song et al., 2011; Kouris et al., 2012; Shadrin et al., 2015). Shadrin et al. (2015) found that heterologous cell fusion promoted cardiomyocyte reprogramming back to a progenitor-like state, and showed that stem cell mitochondria were transferred into cardiomyocytes and persisted in hybrids. The available data concerns mainly bone marrow derived MSCs (BM-MSCs) fusion. It has been reported that in the injured tissues, BM-MSCs can fuse in vivo with differentiated cells and form hybrids with a regenerative potential (Freeman et al., 2015). The bone marrow-derived hybrids were found in many organs, such as the brain, retina, liver, muscle, and gut, where they participated in the reestablishment of tissue function (Acquistapace et al., 2011; Song et al., 2011; Kouris et al., 2012; Freeman et al., 2015).

### MIGRATION AND HOMING – CRUCIAL PROCESSES IN MSCS-BASED THERAPY

#### Overcoming the endothelial barrier

Migration and homing of transplanted cells is a very complicated process that is still under investigation. Depending on the type of tissue source from which the MSCs are isolated, they have their unique, individual ability to migrate and home in a place of damage. These properties of MSCs are crucial for stem cell-based therapies (Pendleton et al., 2013; Chen et al., 2014; Ullah et al., 2015). The directional migration is not a random process but it occurs in response to factors (i.e. chemokines) termed as chemoattractants (Russo et al., 2014). The chemokine gradient released from the injured site recruits transplanted MSCs and provokes them to directly migrate to the target site, which we generally refer to as chemotaxis (Yoon et al., 2016). The mechanism of MSCs migration may vary depending on the method of MSCs
administration. Local transplantation in spatial proximity to the injured site causes the cells to circumvent the long migration route through the vascular system. Intravenous (systemic) injection is favored but accompanied by more adverse events (e.g. pulmonary microembolism) (Bolzte et al., 2015), and requires overcoming of the endothelial barrier. Many reports describe MSCs migration as highly resembling the leukocyte migration model. According to this scenario, systemic homing is a multistep process involving rolling, crawling, adhesion and extravasation (diapedesis). In the right spot the MSCs integrate with endothelial cells, then overcome the endothelial basement membrane and the pericyte sheath, and then continue the interstitial migration towards the target site, that is navigated by the chemokine gradient (Laird et al., 2008; Teo et al., 2012; Schmidt et al., 2013; Ullah et al., 2015).

Factors involved in MSCs migration

Many chemokines and their receptors located on the cells’ surface are involved in the migration of MSCs and their homing at the target site (Cong et al., 2014). The inflammation and inflammatory processes play an important role in MSCs migration (Guan et al., 2018; Su et al., 2018). The stromal cell-derived factor-1 alpha chemokine (SDF-1α) and its receptor (CXCR4) play a crucial role in the inflammatory process. The SDF-1α protein acts as a chemoattractant that mediates the recruitment of circulating MSCs that express the chemokine type 4 receptor. It can also affect expression of other factors, such as the heptocyte growth factor (HGF), stem cell factor (SCF) or Fms-related tyrosine kinase 3 ligand, by which cells are targeted to the place of damage (Kitaoji et al., 2009; Back et al., 2011; Wang et al., 2017; Su et al., 2018). Several research groups have shown that growth factors, such as PDGF, TNF-α, and TGF-β1, have a strong chemoattractive effect on MSCs, which enhances their activity for directional migration (Wang et al., 2017). The studies conducted so far have shown that the damaged tissues inside the body secrete numerous cytokines and express factors (chemoattractants) that guide MSCs to the site of damage.

It turns out that not only the source of stem cells and the number of passages, but also the method of cell isolation and culture can have a relevant impact on the migratory potential of MSCs (Ode et al., 2011). It has been shown that an important issue to consider in the migration studies is the appropriate method of culture and medium supplementation, which allows the cells to optimally grow and release factors that are pivotal during the MSCs migration process after their transplantation (Fu et al., 2019). The MMP matrix metalloproteinases that are necessary for efficiency migration and homing of MSCs play an important role in this process. MMPs belong to the proteolytic enzyme family that participates in degradation of the basement membrane and extracellular matrix (ECM) (Ries et al., 2007). The matrix metalloproteinase-1 (MMP1), acting through the MMP1/PAR1 axis, causes decay of the interstitial collagen type I, II and III (Ho et al., 2009; Pan et al., 2014). It has been proven that upregulation of MMP1 expression leads to a high migration capacity of MSCs. In vitro studies have shown that knockdown of the MMP1 gene in MSCs results in inhibition of their migration capacity (Pan et al., 2014). Thus, cells with high MMP1 expression can be a valuable agent in clinical therapy affecting the possibility of cell engraftment at the target site.

During the MSCs in vitro culture, expression of factors involved in the migration process, such as CXCR4, may significantly decrease (Richter et al., 2017). To prevent this effect, the MSCs culture medium can be supplemented with cytokines that stimulate cells and preserve the potential of these cells for migration and homing. It was observed that addition of a cytokine cocktail containing HGF or SCF to the culture medium resulted in improvement of directional migration of cells after administration, as well as homing at the target site (Shi et al., 2007; Richter et al., 2017; Zhang et al., 2017). The oxygen culture conditions, i.e. hypoxia, may lead to an increase in the CXCR4 protein expression, which consequently promotes and activates the migration process (Liu et al., 2010).

The process of stem cell migration depends on the microenvironment in which they are found. Factors secreted during tissue damage lead to the recruitment of the transplanted cells to this site, which is pivotal from the point of view of cellular therapies (Zhang et al., 2017). The CD44 and CXCR4 proteins contribute significantly to this mechanism. The imprisonment of MSCs in the lungs, liver or spleen after intravenous infusion, constitutes a serious obstacle in cellular therapies. For this reason, therapies often use administration of cells directly at the site of injury (Russo et al., 2014). Nevertheless, stem cell properties allow for efficient migration and colonization at the site of injury. Initiation of the migration process can take place not only through mechanical stimuli (e.g. acute shear stress) but also by activation of receptors located at the MSCs surface, even through factors at a large distance from the target site (Kim et al., 2013; Artemenko et al., 2016). Activated MSCs enter the appropriate signaling pathway which initiates the migration and adhesion processes, and adaptation to the ECM environment (Kim et al., 2013; Russo et al., 2014).

ADHESION MOLECULES – KEY PLAYERS IN MIGRATION AND HOMING OF MSCs

Several classes of cell adhesion molecules mediate interaction between the cell and the substratum. Integrins are the most important among them. They participate in the cell-substratum and cell-cell interactions and are present on almost all human cells (Albelda and Buck, 1990). The MSCs adhesion ability differs due to a different expression of certain integrins, such as α1, α2, α3, α4, α5, α6, αV, β1, and β2 (Semon et al., 2010; Schmidt et al., 2013). FACS analyses of functional characterization of MSCs described integrins αV, α1, α2, α3, α5, and α6, but not α4, β3, β4, β1, and β2 (Uder et al., 2018). MSCs may feature different adhesion properties depending on the tissue source, isolation and culture procedures (Barry & Murphy, 2004; Lin et al., 2006; Rai et al., 2010; Ren et al., 2015; Sanjurjo-Rodriguez et al., 2017; Uder et al., 2018). Many studies demonstrated that β1 integrins are important for the intramyocardial trafficking of MSCs (Ip et al., 2008), and are crucial for the rolling and adhesion of MSCs (Ruster et al., 2006).

Additionally, a study by Ip and others (Ip et al., 2008) suggested that the very late antigen-4 (VLA-4) and vascular cell adhesion molecule-1 (VCAM-1) are expressed in MSCs (Ip et al., 2008). Among them, both the α4 and β1 subunits play a crucial role in VLA-4 (the integrin very late antigen) formation. Nitzsche and others and Chamberlain and others (Chamberlain et al., 2007; Nitzsche et al., 2017) describe the VLA-4 involvement in the endothelial rolling and apprehension at the inflammation sites. Both authors cite the results of experiments confirming the VCAM-1’s molecule role in firm
adhesion of MSCs. In many investigations, blocking the VCAM-1 or VLA-4 mediator, had significantly inhibited adhesion of MSCs to the endothelial cells (D’Ambrosio et al., 1998; Segers et al., 2006). These studies have verified that MSCs bind to endothelial cells in a P-selectin dependent manner. Ruster and others (Ruster’s et al., 2006) showed that rolling of MSCs engages VLA-4/VCAM-1 to provide firm adhesion to the endothelial cells (Ruster et al., 2006).

Moreover, the study of Ruster’s and others (Ruster’s et al., 2006) presents P-selectin involvement in the extravasation cascade – as it has been proven in the MSCs animal model (Ruster et al., 2006). In this study, the P-selectin deficient mice show a decrease in MSCs rolling along the walls of the blood vessels in the ear veins. Similar observations have been also made in the umbilical vein endothelium in vitro model, where MSCs rolling has decreased by neutralizing the P-selectin antibody. The L-selectin expression is described as insignificant in the case of MSCs rolling on endothelium during the first stage of the recruitment process. In comparison to leukocytes, their expression on MSCs is much lower. Additionally, no expression of PECAM-1/CDS1 (platelet/endothelial cell adhesion molecule 1) has been found on MSCs (Bruder et al., 1998; Pittenger et al., 1999; Shur et al., 2002; Ruster et al., 2006), whereas occurrence of hematopoietic cell L-/E-selectin ligand, as well as NCAM1/CDS6 (neural adhesion molecule 1), ICAM2/CDS102 (intercellular cell adhesion molecule 2), MCAM/CD146 (melanoma cell adhesion molecule 2), and ALCAM/CDS166 (activated leukocyte cell adhesion molecule), and ICAM1/CDS54 or ICAM3/CDS50 has been shown. In addition, the P-selectin antigens are not present on the surface of MSCs (Uder et al., 2018). In contrast to the Minguell and others (Minguell et al., 2001) results, Krampera and others (Krampera et al., 2006) described expression not only of VCAM-1 and ALCAM, but also of ICAM-1 and ICAM-3, and in addition that of endoglin/CDS105 (Minguell et al., 2001; Krampera et al., 2006). As it has been previously mentioned, the existing differences in expression may be the consequence of experimental conditions, e.g. cell culture or their source. Segers et al. (2006) described a significant role of VCAM-1 in the animal rat model where they have stimulated the cardiac microvascular endothelial cells and then they have monitored adhesion of MSCs to them (Segers et al., 2006). There is no proven role for the ICAM adhesion molecule in this model.

Regarding the mechanism of the homing process, active surface molecules that are involved in MSCs – endothelium adhesion are in the center of interest. They are believed to be involved in the principal mechanism of this process (Karp & Leng, 2009). As expression of certain chemokine receptors during the extravasation process is widely described, it is still unclear what their exact involvement in this process is.

The presence of CCR2, CCR4, CCR7, CCR10, CXCR5, CXCR6, and CXCR4 is referred to in the literature (Wynn et al., 2004; Ringe et al., 2007; Andreas et al., 2014; Nitzsche et al., 2017). CCR2 participates in organ-specific homing (Belema-Bedada et al., 2008; Guo et al., 2013). In the Smith’s and others (Smith’s et al., 2012) research, chemokines: CXCL12, CXCL13, CXCL16, CCL11, and CCL22 are described as the ligands for the chemokine receptors: CXCR4, CXCR5, CXCR6, CCR3, and CCR4, which have been identified on the MSCs. In this study, the authors show a significantly enhanced transendothelial migration across the bone marrow endothelial cells (Smith et al., 2012). On the other hand, in the research by Ip and others (Ip et al., 2007), CXCR4 is not directly involved in the MSCs transendothelial migration, despite its proven expression on these cells. One of the reasons for such inconsistency in the role of specific cytokines may be rooted in the previously mentioned differences in experimental conditions that vary between the studies, including cell culturing, type of medium, use of serum etc., as well as the isolation procedures (Uder et al., 2018).

Two signaling pathways are established to be of a great importance for the MSCs transendothelial migration. The phosphoinositide 3-kinase (PI3K)/Akt pathway and phosphokinase C (PKC) pathway are described to have a significant role in transendothelial migration. Among the different kinases involved in the MSCs signaling, the integrin-linked kinase (ILK) is indicated. Its role as an intracellular adapter in transmission of signals from the outside to the inside is discussed in the literature (Fig. 2) (Schmidt et al., 2006; Picinich et al., 2010; Widmaier et al., 2012).

**THE INFLUENCE OF OXYGEN CONCENTRATION ON BIOLOGICAL PROPERTIES OF MSCs**

The important issue in the investigation of biological properties of MSC is the microenvironment (niche) in which the cells reside. One of the crucial factors is
oxygen concentration. It should be noted that the standard culture conditions in vitro including 21% O₂ (21 kPa/160 mmHg) are not identical to the in vivo environment, where oxygen concentration is significantly lower, about 2–9%, depending on the vascularization of the tissue and its direct contact with air (Brahimi-Horn & Pouyssegur, 2007; Ward, 2008). In the bone marrow, as a primary source of stem cells (both hematopoietic and mesenchymal), the concentration levels of O₂ have been reported to range from 1.5% to 7% (Spencer et al., 2014). A change in the oxygen level in an in vitro culture leads to alterations in metabolism (secretion of signaling factors – growth factors and cytokines), proliferation (self-renewal), motility (adhesion, migration), and differentiation potential. Consequently, the stemness potential can be lost.

A key factor that plays a crucial role in the regulation of stem cells metabolism is HIF-1α (hypoxia-inducible factor 1 alpha), a transcription factor involved in cellular response to low oxygen availability. HIF-1α is expressed by cells in a hypoxia environment and makes their metabolism pathways similar to those occurring in an in vivo niche (Takubo et al., 2010). These facts explain why the oxygen level should be particularly taken into account during cell expansion for clinical applications.

The results of performed studies have revealed that hypoxia or HIF-1α stabilization have a beneficial effect on MSCs functions, such as proliferation, migration and adhesion, regardless of the tissue type of their origin. Low oxygen concentration favors maintenance of MSCs physiological properties, including proper expression of surface receptors involved in MSCs migration and adhesion (Li et al., 2013; Choi et al., 2016). Song et al. (2009) presented that hypoxia promotes MSCs adhesion to myocardium, thereby increasing their therapeutic potential (Song et al., 2009).

Many studies have also observed a hypoxia dependent effect on the paracrine activity of MSCs. For example, secretion of the vascular endothelial growth factor (VEGF) by AD-MSCs (adipose tissue derived-MSCs) is considerably enhanced under hypoxic conditions, where HIF-1α is more stable (Kang et al., 2014). VEGF, apart from its proangiogenic properties, also stimulates motility of stem cells (Yun et al., 2009).

There is also evidence that the proliferation capacity of MSCs is significantly improved in an environment with low oxygen concentration (Lech et al., 2016; Choi et al., 2017). Some studies suggest that a low oxygen level promotes self-renewal (asymmetric) divisions and inhibits symmetric ones, which precedes cell differentiation (Liu et al., 2012). The reduced oxygen content during stem cell culture has also a beneficial effect on their genomic stability. Lech and others (Lech et al., 2016) have showed that AD-MSCs cultured in reduced oxygen content (5% O₂) maintain a correct karyotype profile compared to cells cultured in normoxia condition. In cultures growing under 21% O₂ concentration numerous karyotyping abnormalities (i.e. chromosomepolyplody and haploid chromosomes) have been observed (Lech et al., 2016).

As detailed above, hypoxia promotes kinetic growth, genetic stability, proper pattern of MSC-specific surface markers, and increases cell lifespan, as well as exerts a positive effect on the paracrine activity and adhesion properties. Thus, conducting cell culture at the physiological oxygen level seems to be more effective and safe in the case of MSCs used in regenerative therapies.

CONCLUSIONS

Mesenchymal stem cells are undoubtedly the future of regenerative medicine. The benefits of their clinical use have been proven in many studies, but development of safe and efficient therapy requires future investigations. The therapeutic potential is a result of their unique properties, including the paracrine activity, directional migration, adhesion and homing at the site of injury. A particular advantage of MSCs when compared to pharmaceutical agents is their capability to secrete a cocktail of bioactive factors in response to circumstances and microenvironmental conditions. It should be noted that the culture conditions and the cell source have fundamental influence on biological properties of MSCs which should be considered in clinical use of MSCs.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

Acquisto AP, Bru T, Lesauh PF, Figuea F, Couder AE, le Coz O, Christov C, Baudin X, Aubert F, Yyou R, Dubois-Randé JL, Rodri-guez AM (2011) Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondrial transfer. Stem Cells 29: 812–824. https://doi.org/10.1002/stem.632.

Albeda S, Buck C (1990) Integrins and other cell adhesion molecules. FASEB J 4: 2868–2880. PMID: 2192985.

An SY, Jang YJ, Lim HJ, Han J, Lee J, Lee G, Park Y, Park SY, Kim JH, Do BR, Han C, Park HK, Kim OH, Song MJ, Kim SJ, Kim JH (2017) Milk fat globule-EGF factor 8, secreted by mesenchymal stem cells, protects against liver fibrosis in mice. Gastroenterology 152: 1174–1186. https://doi.org/10.1053/j.gastreo.2016.12.003.

Andreas K, Stintzing M, Ringé J (2014) Toward in situ tissue engineering: Chemokine-guided stem cell recruitment. Trends Biotechnol 32: 483–492. https://doi.org/10.1016/j.tibtech.2014.06.008.

Artemenko Y, Axiotalis L, Borleis J, Iglesias PA, Devreotes PN (2016) Chemical and mechanical stimuli act on common signal transduction and cytoskeletal networks. Proc Natl Acad Sci U S A 113: e7500–e7509. https://doi.org/10.1073/pnas.1608771113.

Back SJ, Kang SK, Ra JC (2011) In vitro migration capacity of human adipose tissue-derived mesenchymal stem cells reflects their expression of receptors for chemokines and growth factors. Exp Mol Med 43: 596–603. https://doi.org/10.3858/emm.2011.43.10.069.

Barry FP, Murphy JM (2004) Mesenchymal stem cells: Clinical applications and biological characteristics. Int J Biochem Cell Biol 36: 568–584. https://doi.org/10.1016/j.biocel.2003.11.001.

Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T (2008) Efficient homing of multipotent adult mesenchymal stem cells depends on FRCUT-mediated clustering of CCRI2. Circ STEM Cell 2: 566–575. https://doi.org/10.1007/s12920-008-003.003.

Bi B, Schmitz R, Iraisova M, Nishio H, Cantley LG (2007) Stromal cells protect against acute tubular injury via an endothelial effect. J Am Soc Nephrol 18: 2480–2496. https://doi.org/10.1016/S0884-1336(07)60010-8.

Boltez J, Arnold A, Wałczak P, Jolkoñen J, Wagner DC (2015) The dark side of the force – constraints and complications of cell therapies for stroke. Front Neuro 20: 155–171. https://doi.org/10.3389/fnana.2015.00015.

Brabham-Horn MC, Pouyssegur J (2007) Oxygen, a source of life and stress. FEBs Let 581: 3582–3591. https://doi.org/10.1016/j.febslet.2007.06.018.

Briñi AT, Amodea G, Ferreira LM, Milani A, Niada S, Moschetti G, Brahimi-Horn MC, Pouyssegur J (2007) Mesenchymal stem cells reflect their expression of receptors for chemokines and growth factors. Exp Cell Res 313: 155–171. https://doi.org/10.1016/j.yexcr.2015.01.015.

Brahimi-Horn MC, Pouyssegur J (2007) Oxygen, a source of life and stress. FEBs Let 581: 3582–3591. https://doi.org/10.1016/j.febslet.2007.06.018.

Brini AT, Amodea G, Ferreira LM, Milani A, Niada S, Moschetti G, Franchi S, Borsani E, Rodella LF, Panerai AE, Sacerdoti P (2017) Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain. Sci Rep 7: 1–15. https://doi.org/10.1038/s41598-017-09487-5.

Budler SP, Ricalton NS, Boynton RE, Connolly TJ, Jaiswal N, Zaia J, Barry FP (1998) Mesenchymal stem cell surface antigen SB-10 correlates with the presence of active connexin-43 (Cx43) in connexin-43-deficient CB-10. J Histochem Cytochem 46: 749–759.

Burlacu A, Grigorescu G, Rosca AM, Preda MB, Simionescu M (2013) Factors secreted by mesenchymal stem cells and endothelial progenitor cells have complementary effects on angiogenesis in vitro. Stem Cells Dev 22: 643–653. https://doi.org/10.1089/scd.2012.0273.
Chamberlain G, Fox J, Ashton B, Middleton J (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation potential, immunologic features, and potential for homing. Stem Cells 25: 2370–2374. https://doi.org/10.1634/stemcells.2007-0197

Chen G, Yue A, Ruan Z, Yin Y, Wang R, Ren Y, Zhu L (2014) Monitoring the history of human umbilical cord-derived mesenchymal stem cells during long-term culture in serum-free medium. Cell Transfus Bone Marrow Ther 15: 513–521. https://doi.org/10.1186/1944-1042-15-296

Chen X, Xu Y, Zhao J, Zhang Y, Yang R, Xie J, Liu X, Qi S (2014) Conditioned medium from hypoxic bone marrow-derived mesenchymal stem cells enhances wound healing in mice. PLoS One 9: e96161. https://doi.org/10.1371/journal.pone.0096161

Choi W, Koeweling SJ, Lin HJ, Jeong SY, Cai W, Yang YS, Jeon HB, Joon ES (2017) Optimization of culture conditions for rapid clinical-scale expansion of human umbilical cord blood-derived mesenchymal stem cells. Curr Transf Med 6: 38. https://doi.org/10.1186/s40109-017-0168-z

Choi JH, Lim SM, Yoo YI, Jung J, Park JW, Kim GJ (2016) Micro-environmental interaction between hypoxia and endothelial cells controls the migration ability of placenta-derived mesenchymal stem cells via alpha integrin and rho signaling. J Cell Biochem 117: 1145–1157. https://doi.org/10.1002/jcb.25398

Cong Q, Yeh J, Xia XC, Mishina Y, Hao AJ, Li BJ (2014) PDGF-AA promotes osteogenic differentiation and migration of mesenchymal stem cells. Sci Rep 4: 811–820. https://doi.org/10.1038/srep04834

Kitaori T, Ito H, Schwarz EA, Tsutsumi R, Yoshimoto H, Oishi S, Nakano M, Fujii N, Nagasawa T, Nakamura T (2009) Stromal cell-derived factor 1/CXCR4 signaling is critical for homing of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheumatol 60: 813–823. https://doi.org/10.1002/art.24330

Koivusalo NA, Schaefer JA, Hatta M, Freeman BT, Kamp TJ, Kawooya Y, Ogle BM (2012) Directed fusion of mesenchymal stem cells with cardiovoyces via SVG-G facilitates stem cell programming. Stem Cells 30: 4140–4148. https://doi.org/10.1002/stem.12330

Kaur J, Singh KN, Kaur A, Jolly R, Sethi DS, Verma A, Khurana D, Malhotra S, Ahuja R, Sant DS, Jhaveri P (2014) Hypoxic preconditioning advances CXCR4 and CXCR7 expression and mediates chemotaxis of mesenchymal stem cells in lung cancer. Cytokine Growth Factor Rev 25: 397–407. https://doi.org/10.1016/j.cytogfr.2014.04.003

Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells. Cytotherapy The International Society for Cellular Therapy position statement. 2006; 8: 2837.

Koivusalo NA, Schaefer JA, Hatta M, Freeman BT, Kamp TJ, Kawooya Y, Ogle BM (2012) Directed fusion of mesenchymal stem cells with cardiovoyces via SVG-G facilitates stem cell programming. Stem Cells 30: 4140–4148. https://doi.org/10.1002/stem.12330

Kaur J, Singh KN, Kaur A, Jolly R, Sethi DS, Verma A, Khurana D, Malhotra S, Ahuja R, Sant DS, Jhaveri P (2014) Hypoxic preconditioning advances CXCR4 and CXCR7 expression and mediates chemotaxis of mesenchymal stem cells in lung cancer. Cytokine Growth Factor Rev 25: 397–407. https://doi.org/10.1016/j.cytogfr.2014.04.003

Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells. Cytotherapy The International Society for Cellular Therapy position statement. 2006; 8: 2837.

Koivusalo NA, Schaefer JA, Hatta M, Freeman BT, Kamp TJ, Kawooya Y, Ogle BM (2012) Directed fusion of mesenchymal stem cells with cardiovoyces via SVG-G facilitates stem cell programming. Stem Cells 30: 4140–4148. https://doi.org/10.1002/stem.12330

Kaur J, Singh KN, Kaur A, Jolly R, Sethi DS, Verma A, Khurana D, Malhotra S, Ahuja R, Sant DS, Jhaveri P (2014) Hypoxic preconditioning advances CXCR4 and CXCR7 expression and mediates chemotaxis of mesenchymal stem cells in lung cancer. Cytokine Growth Factor Rev 25: 397–407. https://doi.org/10.1016/j.cytogfr.2014.04.003

Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells. Cytotherapy The International Society for Cellular Therapy position statement. 2006; 8: 2837.

Koivusalo NA, Schaefer JA, Hatta M, Freeman BT, Kamp TJ, Kawooya Y, Ogle BM (2012) Directed fusion of mesenchymal stem cells with cardiovoyces via SVG-G facilitates stem cell programming. Stem Cells 30: 4140–4148. https://doi.org/10.1002/stem.12330

Kaur J, Singh KN, Kaur A, Jolly R, Sethi DS, Verma A, Khurana D, Malhotra S, Ahuja R, Sant DS, Jhaveri P (2014) Hypoxic preconditioning advances CXCR4 and CXCR7 expression and mediates chemotaxis of mesenchymal stem cells in lung cancer. Cytokine Growth Factor Rev 25: 397–407. https://doi.org/10.1016/j.cytogfr.2014.04.003

Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells. Cytotherapy The International Society for Cellular Therapy position statement. 2006; 8: 2837.

Koivusalo NA, Schaefer JA, Hatta M, Freeman BT, Kamp TJ, Kawooya Y, Ogle BM (2012) Directed fusion of mesenchymal stem cells with cardiovoyces via SVG-G facilitates stem cell programming. Stem Cells 30: 4140–4148. https://doi.org/10.1002/stem.12330

Kaur J, Singh KN, Kaur A, Jolly R, Sethi DS, Verma A, Khurana D, Malhotra S, Ahuja R, Sant DS, Jhaveri P (2014) Hypoxic preconditioning advances CXCR4 and CXCR7 expression and mediates chemotaxis of mesenchymal stem cells in lung cancer. Cytokine Growth Factor Rev 25: 397–407. https://doi.org/10.1016/j.cytogfr.2014.04.003

Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells. Cytotherapy The International Society for Cellular Therapy position statement. 2006; 8: 2837.

Koivusalo NA, Schaefer JA, Hatta M, Freeman BT, Kamp TJ, Kawooya Y, Ogle BM (2012) Directed fusion of mesenchymal stem cells with cardiovoyces via SVG-G facilitates stem cell programming. Stem Cells 30: 4140–4148. https://doi.org/10.1002/stem.12330

Kaur J, Singh KN, Kaur A, Jolly R, Sethi DS, Verma A, Khurana D, Malhotra S, Ahuja R, Sant DS, Jhaveri P (2014) Hypoxic preconditioning advances CXCR4 and CXCR7 expression and mediates chemotaxis of mesenchymal stem cells in lung cancer. Cytokine Growth Factor Rev 25: 397–407. https://doi.org/10.1016/j.cytogfr.2014.04.003

Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Isolation, culture, and identification of amniotic fluid-derived mesenchymal stem cells. Cytotherapy The International Society for Cellular Therapy position statement. 2006; 8: 2837.
Su P, Tian Y, Yang C, Ma X, Wang X, Pei J, Qian A (2018) Mesenchymal stem cell migration during bone formation and bone disease therapy. Int J Mol Sci 19: 2343–2357. https://doi.org/10.3390/ijms19082343.

Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda F, Kubota Y, Shima H, Johnson RS, Hirao A, Suzumatsu M, Suda T (2010) Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell 7: 391–402. https://doi.org/10.1016/j.stem.2010.06.020.

Texeira FG, Carvalho MM, Sousa N, Salgado AJ (2013) Mesenchymal stem cells: a new paradigm for central nervous system regeneration? Cell Mol Life Sci 70: 3871–3882. https://doi.org/10.1007/s00018-013-1290-8.

Teo GSL, Ankram JA, Martinelli R et al. (2012) Mesenchymal stem cell transmigrate between and directly through tumor necrosis factor-alpha-activated endothelial cells via both leukocyte-like and novel mechanisms. Stem Cells 30: 2472–2486. https://doi.org/10.1002/stem.1198.

Thankamony SP, Saelstein R (2011) Enforced hematopoietic cell E- and L-selectin ligand (HCELIL1) expression primes transendothelial migration of human mesenchymal stem cells. Proc Natl Acad Sci U S A 108: 2258–2263. https://doi.org/10.1073/pnas.1018064108.

Thery C, Bousmac M, Veron P, Ricciardi-Castagnoli P, Raposo G, Garin J, Amigorena S (2001) Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immunol 166: 7309–7318. https://doi.org/10.4049/jimmunol.166.12.7309.

Tsai MJ, Tsai SK, Hu BR, Lai MY, Ryu JM, Song CH, Han HJ (2009) Role of HIF-1alpha in the migration of human mesenchymal stem cells. Cell Mol Life Sci 66: 3871–3882. https://doi.org/10.1007/s00018-013-1290-8.

Tsai MJ, Tsai SK, Hu BR, Lai MY, Ryu JM, Song CH, Han HJ (2009) Role of HIF-1alpha in the migration of human mesenchymal stem cells. Cell Mol Life Sci 66: 3871–3882. https://doi.org/10.1007/s00018-013-1290-8.

Tsai MJ, Tsai SK, Hu BR, Lai MY, Ryu JM, Song CH, Han HJ (2009) Role of HIF-1 alpha in the migration of human mesenchymal stem cells. Cell Mol Life Sci 66: 3871–3882. https://doi.org/10.1007/s00018-013-1290-8.

Uder C, Bruckner S, Winkler S, Tautenhahn HM, Christ B (2018) Integrin-linked kinase at a glance. J Cell Sci 131: 32–49. https://doi.org/10.1242/jcs.20150025.

van Niel G, Porto-Carreiro I, Simoes S, Raposo G (2006) Exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Biomed Sci 13: C317–C326. https://doi.org/10.1007/s40824-017-10971-1.

Vinals GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, Kwong A, Mitsialis SA, Kourambas S (2018) Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. Am J Respir Crit Care Med 197: 104–116. https://doi.org/10.1164/rccm.201706-0925OC.

Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ, Bellantuono I (2004) A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood 104: 2643–2645. https://doi.org/10.1182/blood-2004-02-0526.

Yeoh RW, Lai RC, Zhang B, Tan SS, Yin Y, The BJ, Lim SK (2013) Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. Adv Drug Deliv Rev 65: 336–341. https://doi.org/10.1016/j.addr.2012.07.001.

Yoon D, Kim H, Lee E, Park MH, Chung S, Jeon H, Ahn CH, Lee K (2016) Study on chemotaxis and chemokinesis of bone marrow-derived mesenchymal stem cells in hydrogel-based 3D microfluidic devices. Biomater Sci 20: 25–39. https://doi.org/10.1039/c5bm00706f.

Yun SP, Lee MY, Ryu JM, Song CH, Han HJ (2009) Role of HIF-1alpha and VEGF in human mesenchymal stem cell proliferation by 17beta-estradiol: Involvement of PKC, PI3K/Akt, and MAPKs. Am J Physiol Cell Physiol 296: C317–C326. https://doi.org/10.1152/ajpcell.00415.2008.

Zhang L, Zhou Y, Sun XY, Zhou J, Yang PS (2017) CXCL12 overexpression promotes the angiogenesis potential of periodontal ligament stem cells. Sci Rep 7: 102–115. https://doi.org/10.1038/s41598-017-10971-1.

Zak PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7: 211–228. https://doi.org/10.1089/10763270175062859.

Zwaal RE, Martinova-Mutuchieva L, Adams G, Edwards C, Moss J, Burger JA, Maini RN (2000) Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res 2: 477–488. https://doi.org/10.1186/ar130.