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Review

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

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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 a one of the key factors affecting MSCs properties.

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

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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 IL1b, 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 diabetics. 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-1β, IL-6 and TNF-α levels were elevated in the dorsal root ganglia and the spinal cord (Brini *et al.*, 2017).

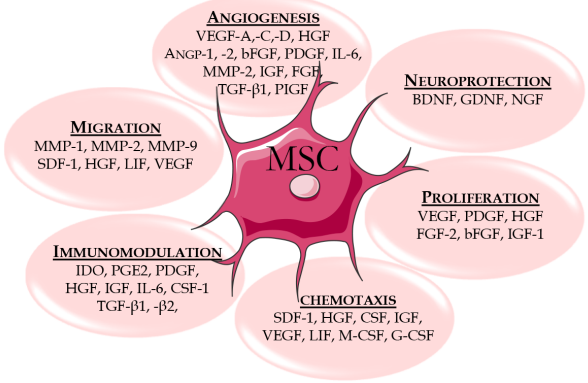

Figure 1. Biological factors secreted by MSCs.

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 (β-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).

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 proteins secreted by MSCs (Ferreira *et al.*, 2018). The cell culture conditions seem to be crucial for the 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 condition (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 (Gnecchi *et al.*, 2005).

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

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

Protein name	Function
GAPDH, enolase 1, aldolase 1, PKM2, PGK1, PDIA3, GSTP1, DPP4, AHCY, TPL1, peroxiredoxins, P4HB, LDH, cyclophilin A, FASN, MDH1 and CNP	Metabolic enzymes
MFGE8 and integrins	Adhesion
HSP60, HSP70, HSPA5, CCT2 and HSP90	Heat-shock proteins
Annexins: I, II, IV, V, VI, VII and X1	Membrane trafficking and fusion
Syntenin, 14-3-3, G proteins, ARF1, CDC42, stomatin, SLC9A3R1, RALA, PDCD6, rack1, mucin 1, EHD1, RAN, PEBP1, MIF, RRAS2, RAC1, NRAS and EHD4	Signal transduction
Vimentin, actins, tubulins, cofilin 1, ezrin, profilin 1, moesin, radixin, myosin, perlecan, THBS1, IQGAP1, keratins, gelsolin, fibronectin 1 and LGALS3BP	Cytoskeletal proteins

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, microRNAs, 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):

- juxtacrine 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 (Rager *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).

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) (Boltze *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 hepatocyte growth factor (HGF), stem cell factor (SCF) or Fms-related tyrosine kinase 3 ligand, by which cells are targeted to the place of damage (Kitaori *et al.*, 2009; Baek *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, α 2, 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, α 2, α3, α5, and α6, but not α4, β, 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; Rui *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

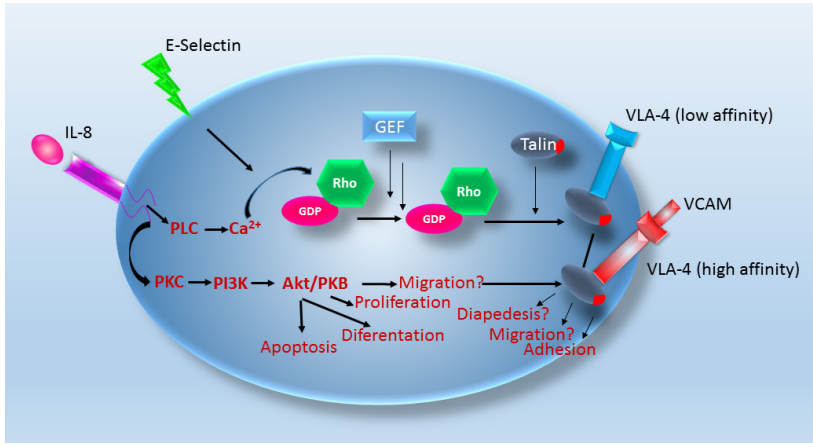

Figure 2. Molecular mechanism of MSCs activation and adhesion to activated endothelium (according to Nitzsche *et al.,* 2017, modified).

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 endothelial *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/CD31 (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/CD56 (neural adhesion molecule 1), ICAM2/CD102 (intercellular adhesion molecule 2), MCAM/CD146 (melanoma cell adhesion molecule 2), and ALCAM/CD166 (activated leukocyte cell adhesion molecule), and ICAM1/CD54 or ICAM3/CD50 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/CD105 (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.

MOLECULAR MECHANISM OF MSCs ADHESION

Hypoxia, acute burns and injury of skeletal muscle, all trigger mobilization of MSCs into peripheral blood (Mansilla *et al.*, 2006; Ramirez *et al.*, 2006; Rochefort *et al.*, 2006). Differences in the cell adhesion molecule pattern, under different culture conditions, may be a starting point for a discussion about the MSCs homing mechanism. At present, there is no detailed and strong data describing MSCs transendothelial migration. Because of existing differences in expression of the cell adhesion molecules between MSCs and well-described leukocytes, there is a need for further detailed investigation of MSCs adhesion, rolling, diapedesis and homing.

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)/Act 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% O_2 (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 O_2 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 *at al.,* 2016) have showed that AD-MSCs cultured in reduced oxygen content (5% O_2) maintain a correct karyotype profile compared to cells cultured in normoxia condition. In cultures growing under 21% O_2 concentration numerous karyotyping abnormalities (i.e. chromosome polyploidy 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.

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