The Role of Autophagy in Mesenchymal Stem Cell-Based Suppression of Immune Response

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Abstract

Mesenchymal stem cells (MSCs) are, due to their capacity for differentiation, immunomodulatory and proangiogenic characteristics, widely used as new therapeutic agents for the treatment of autoimmune, ischemic and degenerative diseases. One of the major barriers for successful transplantation of MSCs is their poor survival after engraftment in the inflamed and hypoxic tissues. Since autophagy regulates survival, differentiation potential, immunomodulatory and proangiogenic characteristics of engrafted MSCs, modulation of autophagy in transplanted MSCs may represent a novel strategy to improve MSCs-based therapy. Until now, modulation of autophagy as a new approach for enhancement of functional characteristics of MSCs has been examined in animal models of multiple sclerosis, osteoporosis, diabetes, myocardial infarction, and graft-versus-host disease. Obtained results suggest that regulation of autophagy may represent a new therapeutic approach that will enhance the efficacy of MSC-based therapy.

Keywords

Autophagy
Mesenchymal stem cells
Immunity
Angiogenesis
Therapy

Abbreviations

3-MA	3-Methyladenine
aGVHD	Acute graft-versus-host disease
Ang-1	Angiopoietin-1
AT	Adipose tissue
ATP	Adenosine triphosphate
BM	Bone marrow
BMT	Bone marrow transplantation
CNS	Central nervous system

DCs	Dendritic cell
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
ECs	Endothelial cells
EGF	Epidermal growth factor
EPCs	Endothelial progenitor cells
FGF-2	Fibroblast growth factor
GIOP	Glucocorticoid-induced osteoporosis
HGF	Hepatic growth factor
HIF-1	Hypoxia-inducible factor 1
HLA	Human leukocyte antigen
HLA-G	Human leukocyte antigen-G
HO-1	Heme oxygenase-1
IDO	Indolamine 2,3-dioxygenase
IFN-γ	Interferon gamma
IL	Interleukin
IL-1Ra	IL-1 receptor antagonist
IL-6	Interleukin-6
LIF	Leukocyte inhibitory factor
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein-1
MHC	Major histocompatibility complex
MI	Myocardial infarction
miRNAs	microRNAs
MMPs	Matrix metalloproteinases
MS	Multiple sclerosis
MSCs	Mesenchymal stem cells
mTOR	Mammalian target of rapamycin
NK	Natural killer
NKT	Natural killer T
NO	Nitric oxide
PD-1	Programmed death 1
PGE2	Prostaglandin E2
PLGF	Placental growth factor
ROS	Reactive oxigene species
TGF-α	Transforming growth factor α
TGF-β	Transforming growth factor-beta
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor alpha
TSG-6	Tumor necrosis factor α-stimulated gene 6
UCB	Umbilical cord blood
VEGF	Vascular endothelial growth factor

8.1. Introduction

Autophagy, as an evolutionary conserved process, plays an important role in homeostasis and embryogenesis, functioning both as a survival and cell death pathway [1]. However, despite its importance in cell physiology, there is little information about the role of autophagy in stem cell survival and function.

Although autophagy is sometimes associated with cell death, it is generally considered to be a survival mechanism because autophagy, unlike apoptosis and necrosis, is activated in conditions of stress represented by hypoxia, nutrient deprivation, and metabolic, oxidative, and proteotoxic stress [2]. Therefore, it has been argued that autophagy is a crucial cellular pathway that regulates development, differentiation, survival, and homeostasis of adult stem cells [3]. Accordingly, herewith, the effects of autophagy on stemness, survival, immunomodulatory and therapeutic characteristics of mesenchymal stem cells (MSCs), the most commonly used adult stem cells in clinical trials, has been emphasized.

8.2. Mesenchymal Stem Cells: New Players in Regenerative Medicine

MSCs (also known as multipotent mesenchymal stromal cells) were first described as fibroblast-like bone marrow populating cells by Friedenstein and coworkers [4]. Due to their immunomodulatory and proangiogenic ability, self-renewal, and differentiation capacity, MSCs are becoming new and promising therapeutic agents for the treatment of autoimmune, ischemic and degenerative diseases [5]. These cells can differentiate into all cell types of mesodermal origin and due to their plasticity, they are able to, in vitro, differentiate into cells of neuroectodermal (neurons, astrocytes, oligodendrocytes, epithelial cells) or endodermal (hepatocytes) origin [6].

Among stem cells, MSCs have several advantages for therapeutic use such as ability to migrate to the sites of tissue injury, strong immunosuppressive effects, better safety after infusion of allogeneic MSCs, and lack of ethical issues, such as those related to the application of human embryonic stem cells [7]. Simple acquisition, rapid proliferation, maintenance of differentiation potential after repeated passages in vitro, minor immunological rejection due to the low surface expression of major histocompatibility complex (MHC) antigens, efficient engraftment and long-term coexistence in the host are the main characteristics of MSCs that enable their therapeutic use [5, 6, 7]. Accordingly, the past decade has witnessed an extraordinary scientific production focused toward the possible clinical use of MSCs in the therapy of autoimmune, ischemic, and chronic inflammatory diseases with very promising findings [5, 6, 7, 8, 9].

As with other multipotent stem cells, MSCs have a high capacity for self-renewal while maintaining multipotency. The exact nature and localization of MSCs in vivo remain undefined, but it appears that they reside in almost all postnatal organs and tissues [8]. Apart from bone marrow (BM), MSCs or MSC-like cells have also been isolated from skeletal muscle, adipose tissue (AT), umbilical cord, blood (UCB) synovium, blood vessel walls, dental pulp, amniotic fluid as well as fetal blood, liver, and lungs [9]. BM, UCB, and AT have been most usually used as sources for the isolation of MSCs [10]. Differences between UCB-MSCs and other MSCs could be observed concerning the success rate of isolating, proliferation capacity and clonality. In contrast to BM-MSCs and AT-MSCs, UCB-MSCs have the highest rates of cell proliferation and clonality and significantly lower expression of p53, p21, and p16, well-known markers of senescence [10].

Diverse antigens have been found on the surface of MSC, but none of them appear to be unique for MSCs [7]. MSCs express CD105 (endoglin, also identified as SH2, a component of the receptor complex of transforming growth factor-beta (TGF-β) involved in proliferation, differentiation, and migration), CD73 (SH3/4, ectoenzyme that regulates the purinergic signaling through the hydrolysis of adenosine triphosphate (ATP)), CD44 (hyaluronan receptor involved in migration), CD90 (Thy-1, regulates differentiation of MSCs), stromal antigen 1 (involved in MSC migration), CD166 (vascular cell adhesion molecule), CD54/CD102 (intracellular adhesion molecule), and CD49b (Integrin alpha-2, involved in adhesion and osteogenic differentiation of MSCs) [10]. The International Society for Cellular Therapy has proposed minimal criteria to define human MSCs: (a) the cells must adhere to plastic in standard culture conditions using tissue culture flasks; (b) more than 95% of the cell population must express CD105, CD73, and CD90 but must lack expression (<2% positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA class II which are expressed on leukocytes, endothelial cells and trombocytes; and (c) the cells must be able to differentiate into adipocytes, osteoblasts, and chondrocytes under standard in vitro differentiating conditions [11].

MSC are a heterogenous population consisting of cells with variable growth potential, distinct morphologic and functional characteristics, but all of them have immunomodulatory and proangiogenic characteristics,

representing a powerful tool in transplantational and regenerative medicine [12].

8.3. Molecular and Cellular Interactions Between MSCs and Immune Cells

The most intriguing aspect of the biology of MSCs is their immunomodulatory potential, such as capacity to suppress T cell proliferation and activation, dendritic cell (DCs) maturation and function, polarization of macrophages, suppression of B cell proliferation and differentiation in antibody producing plasma cells, and attenuation of cytotoxicity of natural killer (NK) and natural killer T (NKT) cells [13].

MSCs modulate proliferation, activation and function of immune cells in cell-to-cell contact (juxtacrine manner) or through the production of soluble factors (paracrine manner) [13]. Interraction between inhibitory molecule programmed death 1 (PD-1) with its ligands PD-L1 and PD-L2 is responsible for MSC-mediated suppression of T-cell proliferation [8, 13]. Despite this mechanism, the capacity of MSC to alter immune response is largely due to the production of soluble factors such as: transforming growth factor-β (TGF-β), hepatic growth factor (HGF), nitric oxide (NO), indolamine 2,3-dioxygenase (IDO), interleukin (IL)-10, IL-6, leukocyte inhibitory factor (LIF), IL-1 receptor antagonist (IL-1Ra), galectins, tumor necrosis factor α-stimulated gene 6 (TSG-6), human leukocyte antigen-G (HLA-G), heme oxygenase-1 (HO-1) and prostaglandin E2 (PGE2) [9, 13]. Through the production of TGF-β, MSCs suppress activation of Jak-Stat signaling pathway and cause the G1 cell cycle arrest, attenuating T, NK, and NKT cell proliferation [9]. IDO, an enzyme induced by proinflammatory cytokines (particularly interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α)) converts tryptophan to kynurenine. The degradation of tryptophan, amino acid that is essential for lymphocyte proliferation, has been suggested to inhibit lymphocyte proliferation [9]. Through the production of IDO, MSCs suppress proliferation of T lymphocytes and attenuates cytotoxicity and cytokine production in NKT cells [14, 15, 16, 17]. Immediately after tissue injury, tissue resident macrophages produce inflammatory chemokines and cytokines which attract MSCs to the sites of wounding where they produce NO that suppress immune cells [16]. Additionally, MSC-derived NO can increase IDO activity and augment MSC-based suppression of immune response [17]. Through the secretion of PGE2, MSCs suppress IL-2 production and the expression of IL-2 receptor on T cells attenuating their proliferation and promote generation of immunosuppressive regulatory T cells [9, 13]. Additionally, MSC-derived PGE2 is responsible for inhibited maturation of DCs and increased alternative activation of macrophages [9].

MSCs modulate function of all immune cells affecting both innate and acquired immunity [13]. MSCs inhibit the division of stimulated T cells by preventing their entry into the S phase of the cell cycle and by mediating irreversible G0/G1 phase arrest [18]. In contrast to the strong inhibitory effects of MSCs on T cell proliferation, there are only relatively minor and reversible effects on T cell effector function, particularly IFN-γ production [19]. Additionally, MSCs do not significantly affect T cell activation (based on CD25 and CD69 surface expression on MSC-primed T cells), does not appear to be antigen specific, works across human leukocyte antigen (HLA) barriers and targets both primary and secondary T cell-driven immune response [19].

MSCs also inhibit the differentiation of monocytes into immature DCs blocking of the monocyte cell cycle at the G0 phase [20]. Cell contact between MSCs and DCs is not required for MSC-based modulation of DC maturation [13]. As a result of cross talk with DCs, MSCs produce soluble factors that attenuate maturation of DCs, downregulate expression of costimulatory molecules and suppress production of cytokines in DCs significantly reducing their ability to stimulate T cells [13].

B cell proliferation is inhibited by MSCs-derived soluble factors [9, 13]. B cell inhibition by MSCs is attributable to blockade of the G0/G1 phases of the cell cycle, similar to what occurs with T cells [21]. MSCs also reduce the expression of chemokine receptors and immunoglobulin production by activated B cells [21]. They do not, however, appear to alter surface molecules involved in stimulatory cell cooperation, such as HLA-DR, CD40 and the B7 costimulators, or to inhibit the production of TNF-α, IFN-γ, IL-4, and IL-10 [21].

MSCs significantly inhibit IL-2-stimulated proliferation of resting NK cells, but only partially impair proliferation of activated NK cells [13, 22]. Cell-to-cell contact and soluble factors such as TGF-β, NO, IDO,

and PGE2 are responsible for this effect [9, 22]. Similarly, in NO and IDO dependent manner, MSCs suppress cytotoxicity and production of inflammatory cytokines in liver NKT cells [17, 23]. MSCs, through the secretion of immunosuppressive factors, suppress inflammatory M1 macrophages and promote their conversion in alternative M2, significantly attenuating macrophage driven inflammation [9, 13, 24].

8.4. Impact of MSCs on Angiogenesis

During ischemic injury, MSCs have been shown to promote angiogenesis through the: (a) secretion of trophic factors; (b) stimulation of endogenous endothelial progenitor cells (EPCs); (c) immune regulation of the microenvironment to enhance survival and proliferation of endothelial cells (ECs) [25].

A broad repertoire of angiogenic factors have been detected in the secretome of BM-MSCs, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, angiopoietin-1 (Ang-1), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), and placental growth factor (PLGF), that enhance angiogenesis [[26], 27, 28, 29, 30]. Additionally, recently, in secretome of BM-MSCs, Cyr61 protein (cysteine-rich, angiogenic inducer 61), an important proangiogenic molecule, was identified [27] indicating its role in MSC-mediated generation of new blood vessels. It is well known that hypoxic conditions, TNF-α and lipopolysaccharide (LPS) upregulate the secretion of angiogenic factors by MSCs in an NF-kappa β dependent manner [29]. Transforming growth factor α (TGF-α), produced after ECs injury, through the activation of MEK/MAPK and the PI3K/AKT signaling pathways, induces production of proangiogenic factors (VEGF, HGF, IL-6, IL-8, and Ang-2) in BM-MSCs [30]. In order to grow, capillaries require degradation of the surrounding extracellular matrix (ECM) to allow endothelial cell sprouting. Matrix metalloproteinases (MMPs) are a group of enzymes that are responsible for the degradation of extracellular matrix proteins [29]. Some of them, including MMP2, MMP9, and MMP14 are secreted by MSCs [29], playing an important role in MSC-dependent modulation of angiogenesis.

Since MSCs can be found in the perivascular space in virtually all organs from where MSCs were obtained, particularly interesting is their interaction with pericytes and EPCs [31, 32]. Pericytes may be considered as vascular MSCs capable of migrating under appropriate stimulation from the MSC vascular niche to the vascular tube where they regulate the neovascularization by secreting cytokines, such as VEGF-A [33, 34]. EPCs stimulate angiogenesis mainly by secreting proangiogenic cytokines (VEGF, HGF, G-CSF, and IL-8) that induce recruitment, proliferation and survival of mature ECs [35]. MSC-EPC interaction relies on both paracrine and cell-to-cell communication [36]. Effects of MSCs and EPCs on angiogenesis are complementary since factors that are produced by EPCs successfully stimulate engraftment and MSC-mediated neovasculogenesis of transplanted MSCs [36]. Intercellular communication between MSCs pericytes and EPCs can also be modulated via the production of microcellular or nanocellular membrane vesicles, which can carry mediators (growth factors, cytokines, lipids, proteins) and genetic information (mRNA, premiRNA, miRNA, tRNA) between cells [37]. The ability of such microvesicles to stimulate angiogenesis has been described both in vitro and in vivo and emerging evidence indicates that microRNAs (miRNAs) play a significant role in MSC-mediated vascular biology and tissue repair [38].

8.5. The Role of Autophagy in the Maintenance of MSC Stemness, Survival, and Function

Appearing evidence indicates that autophagy plays a consistent role in the modulation of proliferation, differentiation and stemness of MSC [3]. Therefore, a great effort has been made trying to evaluate the role of autophagy induced by various extracellular or intracellular stimuli in the maintenance of MSC stemness, survival, and function [3].

In order to maintain their stemness, MSCs actively reduce a deterioration process by establishing low-reactive oxygen species environments [3]. Results from a recent study indicate that autophagy may have an important role in protecting stemness of MSCs from irradiation injury. It has been demonstrated that autophagy induced by starvation or rapamycin can reduce reactive oxygen species (ROS) accumulation-associated DNA damage, therefore maintaining stemness of MSC, whereas inhibition of autophagy leads to ROS accumulation and DNA damage, ultimately resulting in cell death [39].

Several studies investigated the role of hypoxia-induced autophagy for stemness, proliferation and survival of MSCs [40, 41, 42, 43]. Hypoxia-inducible factor 1 (HIF-1) is a transcription factor, which functions as a master regulator of adaptive responses to hypoxia by improving a local microcirculation via its effects on vascular growth [40]. Recent studies have indicated that HIF-1 regulates the autophagy when MSCs are cultured under hypoxic conditions [41, 42, 43]. Hypoxia has been shown to promote BM-MSC proliferation, through the activation of apelin–APJ axis and through the activation of downstream autophagy pathway [41, 42]. Hypoxia-induced apoptosis of MSCs was increased by the autophagy inhibitor 3-methyladenine (3-MA), and decreased by rapamycin, a positive inducer of autophagy, suggesting that the self-eating process might protect MSCs from hypoxia-induced apoptosis. Additionally, atorvastatin, a commonly prescribed statin, could effectively activate autophagy via AMPK/mTOR pathway increasing survival of MSCs under hypoxic conditions [43].

Although results obtained in all these studies [40, 41, 42, 43] strongly indicate beneficial effects of autophagy on stemness and survival of MSCs, opposite findings were recently reported [44, 45]. As demonstrated by Chang and colleagues, BM-MSCs cultured in a medium containing high glucose concentrations have premature senescence, genomic instability and telomere changes [44]. Activation of autophagy, correlated with senescence changes in BM-MSCs and deletion of autophagy-related genes accelerates senescence of MSCs [44, 45]. In line with this data, 3-MA-induced inhibition of autophagy prevents cell death of MSCs [44], suggesting that inhibition and not activation of autophagy was important for survival of MSCs that were cultured in conditions not optimal.

Commitment of transplanted and engrafted MSCs to different lineages is regulated by many cues in the local tissue microenvironment, such as plating density, cell shape, cytoskeleton tension and adhesive, mechanical or structural cellular properties, and is determined by a variety of growth factors [9]. Among these, FGF, epidermal growth factor (EGF) and HGF stimulate proliferation of engrafted MSC and promote their differentiation toward specific cell types [9]. In line with these findings, it has been recently shown that autophagy as well, can play an important role in the commitment of MSC to different lineages, especially toward the osteoblastic and adipogenic lineages [3]. It was demonstrated that undifferentiated MSC exist in a state of arrested autophagy with an accumulation of undegraded autophagic vacuoles and little autophagic turnover, whereas stimulation of osteogenic differentiation leads to a consistent increase in autophagis turnover [46]. Thus, autophagy seems to be of fundamental importance in the control of osteogenic differentiation and this seems to be related to the early mammalian target of rapamycin (mTOR) inhibition and the late activation of the Akt/mTOR signaling axis [3]. On the contrary, during adipogenic differentiation, the alteration in the autophagosome balance led to significant changes in differentiation efficiency of MSCs. Activation of autophagy inhibited adipocyte formation while accelerated fat accumulation was noticed after autophagosome blockade [46].

Autophagy also has an influence on the immunosuppressive function of MSCs. Most recently, Gao and coworkers demonstrated that rapamycin-induced activation of autophagy strengthened the capacity of MSCs to inhibit $CD4^+$ T cell proliferation, whereas 3-MA-induced inhibition of autophagy weakened the immunosuppressive potential of MSCs against effector T helper cells. It seems that autophagy mainly affected production of immunosuppressive TGF-β in MSCs modulating their capacity to inhibit CD4+ T cells. Rapamycin-pretreated MSCs secreted more while 3-MA-pretreated MSCs secreted less amounts of TGF-β when compared with the control MSCs. Also, exogenous addition of TGF-β recovered the immunosuppressive capacity of 3-MA-pretreated MSCs, whereas blocking of TGF-β (by anti-TGF-β monoclonal antibody) significantly reduced the immunosuppressive capacity of rapamycin-pretreated MSCs toward CD4+ T cells, indicating the importance of cross talk between TGF-β signaling and autophagy pathways for MSC-mediated inhibition of T cell-driven pathology [47]. However, completely opposite findings were reported by Dang and colleagues who investigated the role of autophagy in MSC-mediated suppression of CD4+ T cells in experimental autoimmune encephalomyelitis, murine model of multiple sclerosis [48]. They indicated that inhibition and not activation of autophagy is important for enhancement of MSC-mediated suppression of effector T cells. An inhibition of autophagy significantly increased MAPK 1/3 activation in MSCs, which was essential for PGE2-dependent suppression of CD4+ T cells and attenuation of EAE in MSC-treated animals [48].

In addition to its effects of immunomodulatory functions of MSCs, activation of autophagy in tumor-infiltrated MSCs may provide support for the growth of neighboring tumor cells [49]. MSCs, cultured in

serum-free medium, survive prolonged serum deprivation by utilizing autophagy to recycle macromolecules in beclin-1, ATG10, ATG12 and MAP-LC3 dependent manner. At the same time, MSCs with upregulated autophagy-related genes, increased production of antiapoptotic factors (insulin-like growth factor 1, insulin-like growth factor 2, TGF-β, and insulin-like growth factor binding protein 2) that facilitated the survival of surrounding tumor cells enabling rapid tumor growth [49]. Among all these MSC-derived antiapoptotic factors, it seems that TGF-β had the most important role since TGF-β neutralization completely abrogated protective effects of autophagy activated MSC-derived secretome on tumor cell survival [49].

8.6. The Effects of Autophagy on the Therapeutic Potential of MSCs

One of the major barriers for successful transplantation of MSCs is their poor survival after engraftment in the inflamed and hypoxic tissues [3]. Since autophagy regulates survival [40, 41, 42, 43], differentiation potential [46] and immunomodulatory characteristics [47, 48] of engrafted MSCs, modulation of autophagy in transplanted MSCs may represent a novel strategy to improve MSCs-based therapy of autoimmune, ischemic and degenerative diseases [3]. Until now, modulation of autophagy as new approach for enhancement of immunomodulatory properties of MSCs has been examined in animal models of multiple sclerosis (MS), osteoporosis, diabetes, myocardial infarction, and graft-versus-host disease.

MS is an autoimmune disease that is characterized by inflammation of central nervous system (CNS), demyelination, axonal loss, and degeneration. Results obtained in EAE, well established murine model of MS, indicated the crucial importance of myelin-specific T cells in the pathogenesis of this disease. These CD4+ T cells, activated on periphery, migrate and infiltrate into the CNS, where they, through the production of inflammatory cytokines (TNFα, IFN-γ, IL-17A) induce damage of the myelin and axons [50]. MSC-mediated suppression of CNS-infiltrated CD4+T cells has been shown as promising cell based therapy for the treatment of MS [50]. However, only paucity of transplanted MSCs have been noticed in CNS of MSC-treated mice suffering from EAE indicating that modulation of autophagy should be tested as new approach for enhancement of MSC survival in CNS. It is well known that MSCs, engrafted in CNS, enhance autophagy of neighboring neuronal cells having neuroprotective effect [51]. Additionally, in response to inflammatory cytokines (TNF-α and IFN-γ), MSCs that were transplanted in mice with EAE undergo autophagy, as well, by inducing expression of Beclin 1. Activation of autophagy significantly attenuates capacity of MSCs to suppress CD4+ T cells while inhibition of autophagy (by knocking down Beclin 1), significantly improved the therapeutic effects of MSCs on EAE increasing their immunosuppressive effects on $CD4^+$ T cells [48].

Glucocorticoid-induced osteoporosis (GIOP) is a widespread clinical complication of glucocorticoid therapy, and the most common type of secondary osteoporosis [52]. Oral glucocorticoids reduce the proliferation and increase the apoptosis of osteoblasts, prolong the survival of osteoclasts and enhance bone resorption, so the risk of bone fracture is increased in patients that receive glucocorticoid therapy. At the same time, BM-MSCs represent key cellular source for bone repair and regeneration in GIOP patients [52]. Recently, autophagy was determined as an important mechanism responsible for maintenance of bone tissue homeostasis in GIOP due to its effects on survival of BM-MSCs [52]. Glucocorticoid therapy induced autophagy in BM-MSCs as a mechanism of protection from starvation-induced apoptosis. Accordingly, 3-MA-induced inhibition of autophagy reduced proliferation of BM-MSCs and increased apoptosis of BM-MSCs resulting with the reduction in bone mass. These findings strongly suggest that regulation of autophagy should be considered as a new strategy aimed to increase effects of BM-MSC-based therapy in GIOP patients [52].

Due to their immunomodulatory and proangiogenic characteristics BM-MSCs have been widely used as an attractive candidate for cell-based therapy of myocardial infarction (MI) [53]. However, previous studies have revealed that BM-MSCs have undergone an acute death in 1 week after transplantation in the infarcted heart [53]. Since poor viability of engrafted MSCs limits their therapeutic efficiency, new approaches that will enhance viability of transplanted MSCs are urgently needed. A recently published study has shown that survival of MSCs after transplantation in damaged myocardium can be enhanced by drugs like atorvastatin, which activates autophagy via the AMPK/mTOR pathway [41]. Activation of autophagy in MSCs enables their survival under hypoxic conditions [41]. Additionally, apoptosis of BM-MSCs under hypoxic condition was regulated by autophagy and AMPK/mTOR pathway, as well, indicating that activation of autophagy may be useful approach to enhance survival of engrafted MSCs in ischemic myocardium [54].

The possible therapeutic effect of MSCs in diabetes is suggested by their capacity to generate insulin-producing cells in vitro and to normalize hyperglycemia in vivo, in a diabetic animal model [10]. Additionally, several lines of evidence suggested that mainly due to their proangiogenic characteristics, MSCs may be used for the treatment of diabetic complications: lower limb ischemia, polyneuropathy, cardiomyopathy, nephropathy, erectile dysfunction [5]. However, as previously discussed, MSCs transplanted into an ischemic environment have reduced cell survival rates and impaired angiogenic capacity. Therefore, pretreatment of MSCs in vitro has become a primary method to improve their survival and efficiency. Liu J and colleagues found that hypoxic pretreatment and elevated expression of HIF-1-α did not alter phenotype and differentiation potential of MSCs, but managed to significantly enhance their survival by promoting autophagy and by inhibiting apoptosis through the activation of AMPK/mTOR signaling pathway [55]. Furthermore, activated autophagy in MSCs correlated with increased angiogenesis in the lower limbs of MSC-treated ischemic diabetic rats [55], suggesting that induction of autophagy can be a useful approach for enhancement of proangiogenic characteristics of MSCs. Similar conclusions were made by Liu G and coworkers who evaluated induction of autophagy in MSCs as a new approach for the enhancement of their therapeutic potential in the therapy of diabetic erectile dysfunction [56]. Autophagy can be induced through the JNK-mediated phosphorylation or degradation of Bcl-2, which attenuates Bcl-2 dependent inhibition of Beclin-1 [57]. When MSCs engraft in inflammatory microenvironment, ROS induce, at the same time, apoptosis and autophagy in transplanted MSCs through JNK-mediated Bcl-2 degradation. An augmentation of autophagy counteracts apoptosis in MSCs, thus prolonging MSC survival and improving their therapeutic efficacy in the treatment of diabetic erectile dysfunction [56].

Acute graft-versus-host disease (aGVHD) remains a lethal and significant complication in allogeneic bone marrow transplantation (BMT) recipients [58]. MSCs can protect BMT recipients from the lethal aGVHD through the production of immunosuppressive factors (IL-10, TGF-β and IDO) [58]. However, it has to be highlighted that MSCs are not always immunosuppressive [13]. When MSCs are transplanted in the tissue with high levels of inflammatory cytokines, MSCs develop an immunosuppressive phenotype, but when MSCs are engrafted in the microenvironment with low levels of inflammatory mediators, they obtain proinflammatory phenotype, produce large amounts of proinflammatory cytokines and chemokines that stimulate activation and migration of neutrophils and T cells and increase inflammation [13]. These opposite actions may limit therapeutic use of MSCs in the treatment of aGVHD, and the optimization of their immunomodulatory properties can increase safeness of MSC-based therapy of aGVHD. Kim and colleagues demonstrated that rapamycin-induced activation of autophagy in AT-MSCs significantly increased expression of autophagy related genes (ATG5, LC3A and LC3B) that resulted with increased production of immunosuppressive factors (IL-10, IDO, and TGF-β). Additionally, by promoting expansion of T regulatory cells and by attenuating proliferation and effector functions of CD4+ Th17 cells, rapamycin-treated AT-MSCs more efficiently attenuated aGVHD than control AT-MSCs in vivo [58]. Therefore, activation of autophagy can be further explored as new approach for optimization of the immunomodulatory characteristics of AT-MSCs in the cell-based therapy of aGVHD patients.

8.7. Conclusions

Despite the fact that MSCs are, due to their differentiation, immunomodulatory, and proangiogenic properties, widely used for the treatment of autoimmune, ischemic, and degenerative diseases, these cells have undergone an acute death within 1 or few weeks after transplantation in the ischemic microenvironment. Since an augmentation of autophagy significantly enhances viability, immunosuppressive, and proangiogenic characteristics of engrafted MSCs, regulation of autophagy may represent a new therapeutic approach that will enhance the efficacy of MSC-based therapy.

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