Adv Biochem Eng Biotechnol (2013) 130: 19–37
DOI: 10.1007/10_2012_155

Published Online: 20 December 2012

Fate of Intravenously Injected Mesenchymal Stem Cells and Significance for Clinical Application

Beate Wagner and Reinhard Henschler

Abstract Mesenchymal stromal cells (MSCs) have initially been characterized as a fibroblastlike cell population that can be expanded readily in vitro, and is able to support hematopoiesis in vitro and in vivo. By serendipity it was discovered that MSCs can also be administered into the bloodstream. This mode of application formed a major breakthrough in the clinical use of MSCs, because MSC transplantation was found to cure severe immune hyperactivation states such as graft-versus-host disease after allogeneic bone marrow transplantation, or bacterial sepsis. However, MSCs were found difficult to trace and consensus to date is lacking in the scientific community as to where transplanted MSCs end up in the body and which major principles are responsible for the therapeutic effects of MSCs. This chapter gives an overview of the current knowledge on interactions of freshly transplanted MSCs with the cells in the blood stream and the vessel wall, with major organs such as lung, liver, gut, and spleen, and discusses the limitations of the methodologies used to trace transplanted MSCs. The findings will be put into perspective on how therapeutically applied, culture-expanded MSCs may exert beneficial effects.

Keywords Homing · Mesenchymal stem cells · Mesenchymal stromal cells · Therapy · Transplantation

Contents

B. Wagner · R. Henschler (✉)
Department of Transfusion Medicine, Cell Therapeutics and Hemostaseology,
Ludwig Maximilians University München,
Marchioninistrasse 15, 81433 Munich Germany
e-mail: reinhard.henschler@med.uni-muenchen.de

1 Introduction

For more than three decades, MSCs have been found to play fundamental roles in supporting ectopic hematopoiesis [28] and replacement of tissues of mesenchymal origin [55]. The role of MSCs in the replacement of mesenchymal tissues has been revealed by important work by Arnold Caplan and colleagues, among others, who discovered and worked out the details of differentiation regimens of MSCs into a variety of mesenchymal lineages including osteocytic cells, chondroblastlike cells, adipocytic cells, ligamentlike structures, and cells with smooth muscle characteristics [9, 10]. As a consequence, a multitude of clinical trials using topical administration of MSCs has been initiated, mainly in surgical patients [11]. These applications clearly required engraftment of MSCs for their therapeutic effects, including the incorporation of the transplanted cells into tissues. To date, however, topical MSC therapy has not entered clinical routine. This is in some contrast to intravenous application. The intravenous route has gained increasing attention worldwide and has come up with remarkable therapeutic successes in severely ill patients. Starting from early milestones in the clinical development of intravenous MSCs, approaches to reveal the fate of systemically administered MSCs are discussed below.

2 Intravenous Therapeutic Use of MSCs: The Case of Osteogenesis Imperfecta

Intravenously administered MSCs have meanwhile entered clinical studies and are applied in significant numbers of patients for two major indications: to support hematopoietic regeneration or accelerate engraftment in hematopoietic stem cell transplantation, or to cope with severe disorders of immune regulation. These applications had not initially been foreseen during early clinical development of MSCs. Indeed, a ground-breaking observation that facilitated this development was the work of Horwitz and colleagues, performed in a cohort of severely ill children. In the 1990s, they treated children with an inherited monogenetic disease, named brittle bone disease or osteogenesis imperfecta, who lack correctly synthesized collagen type I thus causing formation of fragile bones in the affected children [33]. The protein defect results in retarded growth, severe malformations, and eventually premature death. Horwitz and colleagues had recognized that allogeneic bone marrow transplantation could restore normal bone formation in these children [33]. In as much as collagen synthesis is a typical function of stromal cells that are also contained in the bone marrow graft, these authors reasoned that engrafting of stromal cells could provide the missing enzymatic activity.

Six children who underwent transplantation of allogeneic culture-expanded MSCs showed significant clinical improvement, and engraftment of donor-derived osteoblasts could be demonstrated in bone specimens using microsatellite DNA markers. Next, Horwitz and colleagues replaced uncultured whole bone marrow by culture-expanded MSCs. They compared short-term cultured to longer-term cultured MSCs from the same donor, and used retroviral marking with two different reporters of the transplanted MSCs. In their seminal publication in [34], they showed that gene-marked MSCs also engraft in the diseased children's bone cavities, and that the transplanted children could principally reach growth accelerations equal to the ones transplanted with allogeneic complete bone marrow [34]. The pioneer work of Horwitz' group work provided essential data for the further successful application of MSCs through the intravenous route in other clinical settings.

These authors have in addition made another pivotal observation in the development of cellular therapies using culture-expanded cells: They found that therapeutic success was not seen in all treated patients. They showed that some patients had developed antibodies directed against bovine antigens present on the MSCs grown in media containing fetal bovine serum [34]. Altogether, these studies greatly encouraged subsequent attempts to use MSCs as an intravenously administered cellular therapeutic.

3 Tracking the Fate of Systemically Delivered MSCs

One of the next steps after these discoveries was to investigate the use of MSCs in patients with tumors along with the application of high-dose chemotherapy regimens, in an attempt to alleviate side effects of the chemotherapy. Koc et al. administered around one million MSCs/kg body weight intravenously and demonstrated that the cells were well tolerated in patients with mammary carcinoma [40], for example. Before or parallel to their first clinical uses, intravenously administered MSCs were systematically assessed in a variety of animal models, including mice, rats, baboons, and dogs. Different methods such as radioactive labeling, fluorescent dye labeling, reporter gene transduction, or detection of natural DNA markers such as microsatellite markers of the transplanted cells were used [3, 20, 30, 7, 41]. The studies in small rodents, and similarly also in non-human primates in a noninjury situation, showed that MSCs were distributed to many tissues, however, they were detectable only in minute quantities within the tissues [20, 21]. These and many other follow-up studies using similar methodologies (reviewed in [36] have revealed these major findings: (i) quantification of MSCs is difficult, and if at all, transplanted cells are found at only low absolute levels within tissues. When absolute levels were determined, quantitative recovery was mostly confined to the lungs [45, 46, 78]. (ii) Engraftment of MSCs is mostly transient, and only few studies were able to demonstrate long-term maintenance of intact MSCs. (iii) Systemically applied MSCs can accumulate in certain tissues or tissue areas, for example, in areas of hypoxia or with inflammation. The following paragraphs focus on specific aspects of MSC accumulation within a variety of tissues and under specific pathophysiological conditions. The studies include attempts to decipher the molecular mechanisms by which MSCs may interact with the vessel wall, migrate through tissues, and mediate tropism, and the question whether MSCs may display tissue-specific therapeutic effects.

4 Fate of Intravenously Administered MSCs in the Lungs

The first organs through which intravenously injected MSCs pass are the lungs. The kinetics of human MSCs injected into mice have been determined as a very rapid uptake of >80 % of injected cells within a few minutes after injection and formation of emboli of MSCs in lung vessels, and exponentially falling clearance of the injected cells with a half-life of about 24 h with practically complete elimination within 100 h [45, 46]. The *Alu* sequence assay detecting human DNA did not trace MSCs in any other tissues at significant levels; the MSCs as detected by chromosomal nucleic acid totalled less than 0.1 % of the injected cells. Similarly, the lungs are the tissue with the highest uptake of rodent-derived MSCs, as seen, for example, by the ^{99m}Tc label in rats with induced myocardial infarction [3]. In contrast to human MSCs which rapidly disappear from the (murine) lungs,

murine MSCs, at least in part, have been found to colonize lung tissue. Breitbach et al. [8] demonstrated the long-term incorporation of intact murine MSCs by the later-occurring malignant conversion of the transplanted MSCs. The interaction of transplanted human or murine MSCs with lung endothelial cells has been shown to depend on the suspension medium in which intravenously applied cells are kept pre-transplantation, and occur through an interaction of MSCs with the integrin ligand VCAM-1 [19]. Long-term incorporation of murine MSCs is enhanced by pre-treatment with bleomycin, which induces damage of lung epithelia [51]. MSCs have been shown to exert an anti-inflammatory effect in the lungs by the release of interleukin 1 receptor antagonist [52]. Nemeth et al. [50] in a murine sepsis model observed that MSCs locate in close proximity to macrophages, and induce macrophages to produce anti-inflammatory IL-10 through the release of prostaglandin E. A number of other hints point to a more complex network of cellular interactions, which can influence lymphocytes, for example, by induction of regulatory T cells, or alterations in the functions of monocytic cells as well as neutrophils. More interactions of MSCs with cells of the immune cells are discussed further below. Further investigations will have to elucidate the (likely predominant) role of the lungs enabling direct interactions between intra-vasal or extra-vasated MSCs with, in particular, cells of the immune system.

5 Elucidation of Mechanistic Steps and Dynamics by which MSCs may Interact with Endothelial Cells

The MSC research field is still actively working on elucidating the fate of intravenously transplanted MSCs within tissues over time. Studies have mainly explored two major aspects: which specific adhesion molecules may be involved in interactions of MSCs with endothelial cells, and given their size and the absence of several typical adhesion molecules that are typically present on leukocytes, is the interaction of MSCs with the vessel wall rather a passive or an active process?

5.1 Expression of Major Adhesion Receptors on MSCs Normally Present on Leukocytes

Main candidate receptors for endothelial interactions that are expressed on MSCs include CD44, the alpha4 beta1 integrin, and chemokine receptors such as CXCR4 (reviewed in [36]. The latter can be modulated in their expression, for example, by cytokine stimulation or by culture of MSCs in spheroids [56, 70]. In contrast, E-selectin ligand, L-selectin, as well as beta2 integrins were mainly found to be absent on both human and murine MSCs [36, 56, 60, 62, 63, 65, 70]. Therefore, it

can be expected that, not only due to their enormous cellular volume as compared to blood leukocytes, but also due to the lack of classical adhesion molecules, MSCs may show major defects in their coordinated extravasation behaviour.

5.2 *Evidence for the Involvement of Specific Leukocyte like Interaction Patterns in MSC-endothelial Interaction*

[68] studied the interaction of MSCs that had been pre-treated with tumor necrosis factor (TNF)-alpha to heart endothelium. They observed that blockade of VCAM-1 using function-blocking antibody resulted in a decrease in adhesion of MSCs, indicating that beta1 integrins are actively involved in this process. However, their data were mainly based on in vitro data. [35], in a homing model of murine MSCs in experimental myocardial infarction, demonstrated that alpha4 integrin was required, whereas the chemokine receptor CXCR4 was dispensable for the entry of transplanted cells into ischemic tissue. These findings propose a specific role of beta1 integrins in the homing of MSCs in infarct models. Applying a P-selectin knockout mouse and intra-vital microscopy, [60] showed that human MSCs use a P-selectin ligand that is not PSGL-1 to affect interaction of MSCs with endothelial cells in collection venules of the murine ear. [62, 63] went further and engineered human MSCs by a specific fucosylation procedure, which do not naturally bind E-selectin at high shear forces, to alter CD44 epitopes to express functional E-selectin ligand activity, and rendered them fully responsive to E-selectin expressed on HUVEC endothelial cells in vitro and on bone venules in vivo. This alteration highly promoted MSC localization into bone tissue. In further work, they suggested that these engineered MSCs mediate their arrest on endothelial cells via capturing E-selectin, followed by ligation of alpha4 beta1 integrin [75].

The ability of human MSCs to adhere reversibly to TNF-alpha pre-stimulated endothelial cells was compared to human CD34 + hematopoietic progenitor cells or freshly isolated human blood mononuclear cells in a flow chamber model employing integrins alpha4 beta1 and its ligand, vascular cell adhesion molecule (VCAM)-1 [60]. In contrast to CD34 + cells and lymphocytes which for the most part reversibly bound to HUVEC endothelial cells, MSCs were found almost unable to dissociate at increased shear stresses at any significant rate. This indicates deficiencies in the dynamics of endothelial cell binding of MSCs.

6 Direct Evidence for a Co-ordinated Extravasation of MSCs

If MSCs would successfully interact with endothelial cells, they are expected to be found in tissue sections. Although numbers of transplanted MSCs in the tissues are low (a few million cells are maximally injected into a mouse, the per kg dose for

humans in phase I/II studies was mostly about one million per kg body weight), [60] described intact MSCs in interstitial areas within the mouse liver. [62, 63] found that the engineered MSCs that express functional E-selectin ligand activity accumulate in bone marrow sinusoidal vessels and remain adjacent to endothelial cells, but within 24 h slowly locate into interstitial areas outside the vessels. This confirms the principal possibility that MSCs can extravasate. However, in both models, functional readouts such as a regenerative function of the MSCs were not investigated.

7 Direct Evidence for Other Fates of MSCs within the Vasculature

Intra-vital microscopy allows us to follow the fate of injected cells more closely. It has been performed in mice, partly after intra-arterial injection [29, 76], or after intravenous administration [78]. In the studies after intra-arterial injection, reappearance of the cells given originally as a bolus has been reported, indicating that they can passage the lungs. Using the cremaster muscle model, [29] observed that blood flow and microcirculation were impaired after injection of MSCs, and that MSCs obstructed small vessels. In addition, lung emboli were also reported. Occlusion of vessels and entrapment of injected MSCs at the pre-capillary level were also reported by [76]. Within a period of three days, in situ cell death of MSCs caught in the microvessels was observed, but also the integration of transplanted cells into the vessel wall. [78] also reported a clear risk of vascular occlusion after intravenous injection in the myocardial infraction model.

In contrast to these rather discouraging findings, studies in murine experimental autoimmune encephalitis also employed intra-vital microscopy and revealed accumulation of transplanted cells in inflammatory foci using bioluminescence, and showed a role of alpha4 integrins in this process [18]. Moreover, GFP+ transplanted MSCs have been localized to lymph nodes and to spleen at increased amounts. Although absolute numbers of transplanted MSCs have not been determined, these results indicate that active inflammation may switch the fate of transplanted MSCs from unspecific entrapment to specific recruitment.

The ability of MSCs to break down interstitial matrix (e.g., by gelatinases) has been demonstrated in mouse myocardium after alpha4 beta1, VCAM-1 mediated adhesion, and transendothelial migration by in situ zymography, and involves matrix metalloproteinase (MMP)-2 [73].

Still, any specific or unspecific clearance pathway for intravenously injected circulating MSCs has so far not been determined.

8 Systemic Reactions after MSC Transplantation

The studies using intra-vital microscopy and intra-arterial delivery of MSCs have already indicated systemic hemodynamic reactions. Walczak et al. using MSCs labeled with superparamagnetic iron oxide and laser Doppler measurements along with MRI imaging, confirmed rheologic perturbations, occlusion of vessels, and an increased mortality in the cell-treated group [78].

In contrast, intravenous infusion of 1–3 × 10E6 culture expanded MSCs/kg was clinically well tolerated in a study comprising 44 patients after hematopoietic stem cell transplantation. No adverse events were detected, but a slight drop of about 15 % in platelet counts was noted as well as a fivefold increase in the coagulation marker TAT (thrombin–anti-thrombin complex) and the anaphylatoxin C3a in serum [49]. In vitro exposure of MSCs to freshly drawn human whole blood in a closed circuit system demonstrated a potential of MSCs not only to decrease platelets but also circulating granulocytes and monocytes, except lymphocytes. This points to an interaction of MSCs with circulating cells. These effects were termed "instant blood-mediated inflammatory reaction" (IBMIR) and found to be proportional to the expression of tissue factor on human MSCs and the passage number of MSCs, and varied between individual donors. IBMIR, which was initially described after pancreatic islet and hepatocyte injection, is thought to be based on the activation of the complement and coagulation systems, and may to some extent interfere with clinical efficacy and safety of MSCs as cellular therapeutics.

On the other hand, MSCs have also been found to be able to respond to complement factors, and to acquire complement factors on their cell surface [48, 67], indicating that activation of systemic inflammation and of coagulation may influence the fate of intravenously delivered MSCs and vice versa.

9 Engraftment of MSCs into Bone Marrow

Bone marrow has been the main origin of MSC preparations that have been investigated in early pre-clinical and clinical work of intravenous administration regimens. Hence, it was of interest to see whether these cells could reach their tissue of origin. Work in the course of establishment of autologous and allogeneic bone marrow transplantation protocols had already shown that stromal cell types, such as colony-forming units-fibroblast (CFU-F), generally tended not to engraft along with the hematopoietic stem cells [72]. Until recently, this finding was hardly challenged and has been confirmed also for stromal cells that have been formally qualified as MSCs [17, 58]. Rombouts et al. showed in kinetic studies of outgrowing MSCs that culture time induced an engraftment defect of MSCs into bone marrow [59]. However, the work of Horwitz et al. [34] demonstrated that MSCs engraft in bone marrow of children with osteogenesis imperfecta, because they were able to grow donor-type MSCs from bone marrow of transplanted children. On the other hand,

[39] did not find engraftment of culture-expanded MSCs in patients with Hurler syndrome or metachromatic leukodystrophy. Direct evidence of bone marrow engraftment of human MSCs was recently shown to depend on the presence of functional integrity of a CD44 epitope (hematopoietic cell E selectin ligand) that was biotechnologically expressed on MSCs [62, 63]. Normal culture-expanded MSCs display only minimal levels of E-selectin binding activity, which may explain the low or undetectable numbers of unmanipulated MSCs found in bone marrow following intravenous injection, both after bone marrow transplantation or after high-dose chemotherapy. Follenzi et al. [25] recently showed that mice suffering from hemophilia lacking coagulation factor VIII, upon transplantation of normal healthy bone marrow show engraftment not only of hematopoietic cells, but also of sub-endothelial mesenchymal stromal cells which contributed to produce functional factor VIII. This also points to engraftment of at least some functional MSCs in the course of bone marrow transplantation, at least when performed in certain enzyme deficiencies.

Still, to date, no substantial donor-type contribution of any intravenously transplanted MSCs to hematopoiesis, either within the bone marrow, or at ectopic sites such as the kidney has been established for MSCs or analogous cell types [12, 13, 28, 42, 61, 66].

10 Role of Local Cues, Including Inflammation, Ischemia, and Previous Irradiation Influencing the Fate of MSCs

In the classical concept of extravasation of circulating immune cells into tissues, local cues mediate alterations in the expression of adhesion molecules on endothelial cells, such as selectins, integrin ligands, and chemokines, This way, circulating cells that are marginalized can increase contact time with endothelia, for example, through tethering and rolling interactions, and arrest and finally transmigrate through the vessel wall into tissues. If this would hold true for MSCs, one would expect an increased accumulation of MSCs in inflamed or ischemic tissues, as observed with phagocytes or cells or lymphocytes. In several murine or rat models of myocardial infarction, MSCs have been found to accumulate preferentially in areas of ischemia (e.g., [14, 65]. Zhang [84] have demonstrated a link between expression of the chemokine, stromal-derived factor (SDF)-1 and local accumulation of MSCs. Belema-Bedada et al. [6], using a transgenic model of the monokine-CC-chemokine ligand (CCL)-2 expressed under a cardiac-specific promotor have observed that intravenously injected MSCs carrying fluorescence markers accumulate selectively in the heart. They also showed that migration to the myocardium involves certain components of the intracellular signaling pathway of G protein-coupled receptors, pointing to the ability of MSCs to respond in a co-ordinated way to chemokines presented on cardiac vessel endothelia. However, the model also includes chemoattraction of circulating monocytes into the myocardium, but the influence of additional signals by monocytes

cannot be ruled out. Together, these data demonstrate an ability of MSCs to enter tissue in the presence of specific environmental and inflammatory cues.

Kraitchman et al. [41] have confirmed accumulation of intravenously injected MSCs into myocardial infarction areas using a radioimaging tracer and single-photon emission computer tomography in a canine model. Some studies trying to trace MSCs at later stages after infarction tend to find markers of the transplanted cells in differentiated, newly regenerated cardiomyocytes (e.g., [79]. However, it is unclear to which cell and tissue types the homed MSCs may directly contribute, whether cell fusion is also involved, or whether in some cases also only artifacts were measured. There are also studies that have failed to detect any homed MSCs in cardiac tissue in the longer term (e.g., [43]).

In favor of some engraftment and tissue incorporation of intravenously transplanted MSCs are studies in inflammatory bowel disease models. Parekkadan et al. [53] have traced MSCs in a murine chemically induced colitis model. They demonstrated the presence of the live cell label not only in the lungs, spleen, and gut of the affected animals [53]. Sasaki et al. [64] have assessed whether MSCs may differentiate into skin cells including keratinocytes, and possibly contribute to wound repair in a mouse model using intravenously injected green fluorescence protein (GFP) transgenic MSCs. They found GFP-positive cells associated with specific markers for keratinocytes, endothelial cells, and pericytes. They suggested a chemokine CCL21-mediated entry mechanism. Although there is evidence of preferential attraction of the intravenously injected MSCs into wounded versus nonwounded skin, numbers of detected cells in the wounded skin areas were low.

MSCs have been demonstrated to migrate into inflamed brain tissue. Wu et al. in a murine stroke model validated MSC migration into ischemic areas after intravenous delivery [81]. Yilmaz et al. [83] confirmed and extended these findings by providing evidence that intravenously administered MSCs that enter ischemic areas in the brain are recruited through endothelial expressed P- and E-selectin, via CD44 present on the MSCs. In a rat brain ischemia model, [80] showed that intact MSCs arrive in ischemic zones and deliver neurotrophic factors at a greater rate when they have been exposed to hypoxia before injection. This correlated with increased expression of chemokine receptor CXCR4 on the MSCs, or the flk-1 and the erythropoietin receptors, and at the same time downregulation of pro-inflammmatory regulators in the MSCs. Miroglia activity was suppressed in animals after MSC therapy, and NeuN-positive and Glut1-positive cells were increased, underscoring the beneficial effects of intravenous delivery of MSCs.

Taken together, MSCs can migrate into ischemic and pro-inflammatory regions in certain disease stages in murine models. Generally, mostly short-term actions are reported, and long-term persistence of MSCs is not reported. Also, only indirect evidence is available to indicate whether the MSCs remain intact cells in their target environments. A quantification of the amount of active MSCs within a lesion, compared with the number of originally injected MSCs, is also not available. Still, these data argue in favor of locally acting, homed MSCs in a part of the investigated pathologies.

11 Influence of Local Irradiation on the Fate of Intravenously Transplanted MSCs

Francois et al. [26] demonstrated in mice that both total body irradiation and also local irradiation (e.g., on abdomen or legs) affected the distribution of hMSCs after IV infusion in NOD/SCID mice as compared to untreated animals. Without irradiation, intravenously infused hMSCs were found only in minimal amounts exclusively in the lung, bone marrow, and muscles. Fifteen days after the above-mentioned irradiation procedures, radiation-induced damage of tissues in the irradiated regions was confirmed by histological examination. TBI-treated animals exhibited higher absolute numbers of hMSCs in the brain, heart, bone marrow, and muscles. Moreover, selective radiation of limbs or the abdomen yielded a higher hMSC engraftment in the exposed field. More hMSCs were detected in the exposed skin, quadriceps, and other muscles than with TBI alone or additional abdominal irradiation. hMSC engraftment outside the locally irradiated regions was also increased, arguing for both local and systemic effects of irradiation for MSC engraftment. Long-term engraftment was, however, not investigated. Sémont et al. [69] in an additional study specifically investigated the engraftment, but also the efficacy of MSCs in a model of radiation-induced gastrointestinal tract failure. They demonstrated accelerated recovery in the group receiving hMSC in immunodeficient mice, with decreased apoptosis of epithelial cells and increased proliferation within the small intestinal mucosa. However, the transplanted MSCs were not detected at significant amounts.

12 Homing and Engraftment of MSCs into Tumors

Tumors of different kinds inherently harbor altered microenvironments with major alterations in blood flow, blood vessel structure, immune cell activation, and accumulation of trophic factors; in these processes, endogenous local or blood-derived mesenchymal stromal cell types are thought to play a major role [24]. Therefore, tumor tissue may represent a potential target for homing of intravenously injected MSCs.

Several studies have recently highlighted the fact that MSC indeed can home into tumors and that they can have both beneficial and also unwanted effects. Direct evidence for the migration of intravenously transplanted MSCs into tumors was provided by lentivirus-transduced MSC expressing eGFP in cells isolated from human orthotopic pancreatic cancer xenografts in nude mice using intra-vital microscopy [4]. Microscopical studies confirmed the interaction of MSCs with endothelial cells of blood vessels.

A more sophisticated method to detect homing of MSCs into tumors was recently developed by insertion of a trifunctional chimeric reporter into human adipose tissue-derived MSCs and a chimeric reporter into human glioblastoma

cells [1]. Because both cell types expressed luciferase reporters they could be measured noninvasively by bioluminescence both in vitro and in vivo in a SCID mouse model. Although MSCs were implanted here and not intravenously injected, the study provides evidence for a concept that treatment with ganciclovir (GCV) activates a suicide mechanism in tumor-resident MSCs, resulting in tumor regression by a factor of 10E4 relative to controls. Using a luciferase reporter regulated by an endothelial-specific (PECAM, platelet/endothelial cell adhesion molecule) promoter and in vivo BLI to detect MSC differentiation, a mechanism was elucidated: implanted MSCs homed to tumor vessels, where they differentiated to endothelial cells [1].

12.1 Pro-Tumorigenic Effects of Tumor Engrafted MSCs

Enhanced angiogenesis as a mechanism of tumor promotion by MSCs was confirmed in vitro and in vivo using a murine BM-derived MSC line against B16 melanoma cells expressing LacZ (B16-LacZ) and Lewis lung carcinoma (LLC) [74]. Both co-culture with MSCs and treatment with MSC-conditioned media led to enhanced growth of tumor cells, although the magnitude of growth stimulation in co-cultured cells was greater than that of cells treated with conditioned media. Co-injection of tumor cells and MSCs into syngeneic mice led to increased tumor size compared with injection of tumor cells alone. Consistent with a role for neovascularization in MSC-mediated tumor growth, tumor vessel area was greater in tumors resulting from co-injection of tumor cells with MSCs than in tumors induced by injection of cancer cells alone. Co-injected MSCs directly supported the tumor vasculature by localizing close to vascular walls and by expressing the endothelial marker CD31. CCL25 was identified as a major chemoattractant for MSCs which was produced by multiple myeloma cells and has been made responsible for growth support of multiple myeloma cells through MSCs [82], providing a rationale to engineer chemokine receptors on MSCs, tailoring them towards an anti-tumor response profile.

12.2 Anti-Tumor Efficacy of MSCs

In a nude mice model, interleukin-12 (IL-12) expressing MSCs was injected intravenously to treat established Ewing sarcomas [22]. Although transplanted MSCs were not directly identified, the ongoing secretion of IL-12 in the tumor microenvironment strongly suggested homing of the injected MSCs to the tumor sites. In addition, growth suppression of the Ewing sarcomas was observed. [37] investigated potential phenotypes of tumor-associated MSCs by multicolored tissue transplant procedures in mice [37]. In syngeneic ovarian and breast cancer

subpopulations they showed tumor-associated fibroblasts (TAFs) originated from MSCs located in the bone marrow, whereas most vascular and fibrovascular stroma (pericytes, α-SMA(+) myofibroblasts, and endothelial cells) were recruited from neighboring adipose tissue. These data form a basis that intravenously injected MSCs follow a path that is already established for endogenous MSCs, circulating to the tumor through the bloodstream. Grisendi et al. [32] demonstrated that this process implies epithelial/endothelial mesenchymal transitions in situ, or occurs through circulating pools of fibroblasts deriving from mesenchymal progenitors. These findings explain the apparent tumor tropism of MSCs, making them an attractive tool in tumor therapy.

There are some promising approaches to using MSCs as a tumor-suppressing cell therapy after incorporation of Paclitaxel, a widely used anti-cancer drug that also inhibits endothelial cell proliferation. Co-injection of tumor cells and a Paclitaxel-loaded MSCs cell line showed the anti-tumor efficacy of this approach [54].

13 Tracing of MSCs In Vivo that may Induce Alterations of the Immune System

MSCs have been shown to affect the immune system and also affect systemic administration in many ways (reviewed in [44]. However, tracing of MSCs has been cumbersome. As already indicated by Horwitz et al. for patients, development of antibodies against allogeneic MSCs, mostly against bovine antigens after ex vivo culture in fetal bovine serum-containing media, has given a good explanation of why MSCs are eliminated from transplanted humans or baboons (Horwitz et al. [5, 34]. The concept of locally acting MSCs to control immune reactions has been visualized in the lungs in the sepsis model of Nemeth et al. [50], but otherwise few MSCs have been found both in mice and in patients; Studies in mice [57, 31] found little evidence of the transplanted MSCs, as did Lee et al. in the TSG-6-mediated myocardial infarction mouse model [45, 46]. The same applied to patients who had received MSCs in the course of severe graft-versus-host disease, in whom it was very rarely possible to trace transplanted MSCs [77].

14 Interactions of i.v. Transplanted MSCs with Other Cell Types

Recently, the first studies have been published that describe responses in distinct immune cell types after intravenous application of MSCs. Although not all studies provide direct evidence for a cellular contact between the MSCs and other cells of

the immune system, direct interactions between these cell types could be visualized in vitro, pointing to a realistic possibility that the interactions also occur in vivo. One study by Chiesa et al. [15] has visualized the almost complete cessation of migrating dendritic cells (DCs) in mice after intravenous delivery of MSCs. Using an in vitro system they show that murine MSCs inhibit DCs through toll-like receptor (TLR) 4. Mechanistic studies using mixed lymphocyte reactions co-culture studies with monocytes and hMSCs revealed a unique immunophenotype of alternatively activated human monocytes being CD206-high, IL-10-high, IL-6-high, IL12-low, and TNF-alpha-low [38]. The immunosuppressive potential of MSCs has been shown to depend on the inducibility of indoleamine 2,3-dioxygenase (IDO) [27]. Other work has identified MSC-secreted prostaglandin E2 as a main mediator of inflammation [47]; Nemeth et al. [50] in their sepsis model have revealed that intravenously administered MSCs use PGE2 as a mediator. This work put into the middle of its central hypothesis a direct influence of MSCs and macrophages in the lung. However, overall, direct interactions have rarely been demonstrated between i.v. injected MSCs and monocytes/macrophages [71].

Recent work by Akiyama et al. [2] has elucidated a role for fas ligand expressed on MSCs, by transiently inducing apoptosis in T cells. It has been shown to involve the secretion of MCP-1 by MSCs, which recruits T cells to apoptosis. The apoptosing T cells activate macrophages to produce TGF-beta, increasing regulatory T cells and thus promoting immune tolerance. More or less direct actions of MSCs on immune cells also involve the secretion of anti-inflammatory protein TSG-6 by activated MSCs which in a zymosan-induced mouse peritonitis model decreased TLR2/NF-κB signaling in resident macrophages [16].

15 Other Fates of MSCs and Outlook

Surprisingly, ectopic tissue formation was not found after systemic administration of MSCs. Also, fusion of MSCs, as has been shown to occur during tissue culture (e.g., in the presence of epithelial cells; [23], has not been regularly observed after MSC administration through the intravenous route. In summary, the terminal fate of the bulk of intravenously injected MSCs therefore remains elusive, inasmuch as studies have generally only been able to detect small amounts of injected cells. An exception is the approximately 80 % of MSCs that have been found transiently in the lungs of mice by [45, 46]. A natural clearance pathway for circulating MSCs has not yet been established. Future work will have to continue to trace transplanted MSCs, involving more quantitative assessments, in order to reveal what we do not really know about these scientifically and clinically fascinating cells.

References

1. Alieva M, Bagó JR, Aguilar E, Soler-Botija C, Vila OF, Molet J, Gambhir SS, Rubio N, Blanco J (2012) Glioblastoma therapy with cytotoxic mesenchymal stromal cells optimized by bioluminescence imaging of tumor and therapeutic cell response. PLoS One 7(4):e35148 (Epub 2012 Apr 17)
2. Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S (2012) Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell 10(5):544–555 (Epub 2012 Apr 26)
3. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH et al (2003) Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation 108:863–868
4. Beckermann BM, Kallifatidis G, Groth A, Frommhold D, Apel A, Mattern J, Salnikov AV, Moldenhauer G, Wagner W, Diehlmann A, Saffrich R, Schubert M, Ho AD, Giese N, Büchler MW, Friess H, Büchler P, Herr I (2008) VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br J Cancer 99(4):622–631 (Epub 2008 July 29)
5. Beggs KJ, Lyubimov A, Borneman JN, Bartholomew A, Moseley A, Dodds R, Archambault MP, Smith AK, McIntosh KR (2006) Immunologic consequences of multiple, high-dose administration of allogeneic mesenchymal stem cells to baboons. Cell Transplant 15(8–9):711–721
6. Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T (2008) Efficient homing of multipotent adult MSCs depends on FROUNT-mediated clustering of CCR2. Cell Stem Cell 2:566–575
7. Bensidhoum M, Chapel A, Francois S, Demarquay C, Mazurier C, Fouil- lard L, Bouchet S, Bertho JM, Gourmelon P, Aigueperse J, et al (2004) Homing of in vitro expanded Stro-1- or Stro-1 + human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. Blood 103:3313–3319
8. Breitbach M, Bostoni T, Roell W, et al (2007) Potential risk of bone marrow cell transplantation into infarcted hearts. Blood 110:1362–1369
9. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98:1076–1084
10. Caplan AI (2009) Why are MSCs therapeutic? new data: new insight. J Pathol 217:318–324
11. Caplan A, Correa D (2011) The MSC: an injury drugstore. Cell Stem Cell 9:11–15
12. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425:841–846
13. Chen Y, Jacamo R, Shi YX, Wang RY, Battula VL, Konoplev S, Strunk D, Hofmann NA, Reinisch A, Konopleva M, Andreeff M (2012) Human extramedullary bone marrow in mice: a novel in vivo model of genetically controlled hematopoietic microenvironment. Blood 119(21):4971–4980 (Epub 2012 Apr 5)
14. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M (2001) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke 32:1005–1011
15. Chiesa S, Morbelli S, Morando S, et al (2011) Mesenchymal stem cells impair in vivo T-cell priming by dentritic cells. Proc Natl Acad Sci USA 108:17384–17389
16. Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ (2011) Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan- induced mouse peritonitis by decreasing TLR2/NF-KB signaling in resident macrophages. Blood 118:330–338
17. Cilloni D et al (2000) Limited engraftment capacity of bone marrow-derived mesenchymal cells following T-cell-depleted hematopoietic stem cell transplantation. Blood 96:3637–3643

18. Constantin G, Marconi S, Rossi B, Angiari S, Calderan L, Anghileri E, Gini B, Bach SD, Martinello M, Bifari F, Galiè M, Turano E, Budui S, Sbarbati A, Krampera M, Bonetti B (2009) Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. Stem Cells. 27(10):2624–2635
19. Deak E, Rüster B, Keller L, Eckert K, Fichtner I, Seifried E, Henschler R (2010) Suspension medium influences interaction of mesenchymal stromal cells with endothelium and pulmonary toxicity after transplantation in mice. Cytotherapy 12(2):260–264
20. Devine SM, Bartholomew A, Mahmud N, Nelson M, Patil S, Hardy W, Cord S, Terry H, Chung T, Stock W et al (2001) Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. Exp Hematol 29:244–255
21. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood 101:2999–3001
22. Duan X, Guan H, Cao Y, Kleinerman ES (2009) Murine bone marrow-derived mesenchymal stem cells as vehicles for interleukin-12 gene delivery into Ewing sarcoma tumors. Cancer 115(1):13–22
23. Ferrand J, Noël D, Lehours P, Prochazkova-Carlotti M, Chambonnier L, Ménard A, Mégraud F, Varon C (2011) Human bone marrow-derived stem cells acquire epithelial characteristics through fusion with gastrointestinal epithelial cells. PLoS One 6(5):e19569
24. Fakhrejahani E, Toi M (2012) Tumor angiogenesis: pericytes and maturation are not to be ignored. J Oncol 2012:261750 (Epub 2011 Oct 9)
25. Follenzi A, Raut S, Merlin S, Sarkar R, Gupta S (2012) Role of bone marrow transplantation for correcting hemophilia A in mice. Blood 119(23):5532–5542 (Epub 2012 Feb 24)
26. Francois S, Bensidhoum M, Mouiseddine M, Mazurier C, Allenet B, Semont A, Frick J, Sache A, Bouchet S, Thierry D et al (2006) Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells 24:1020–1029
27. Francois M, Romieu-Mourez R, Li M, Galipeau J (2011) Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther 20:187–195
28. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV (1974) Stromal cells responsible for transferring the micro- environment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 17:331–340
29. Furlani D, Ugurlucan M, Ong L, Bieback K, Pittermann E, Westien I, Wang W, Yerebakan C, Li W, Gaebel R, Li RK, Vollmar B, Steinhoff G, Ma N (2009) Is the intravascular administration of mesenchymal stem cells safe? mesenchymal stem cells and intravital microscopy. Microvasc Res 77(3):370–376 (Epub 2009 Feb 26)
30. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI (2001) The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs 169:12–20
31. Gonzalez-Rey E, Anderson P, González MA, Rico L, Büscher D, Delgado M (2009) Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 58:929–939
32. Grisendi G, Bussolari R, Veronesi E, Piccinno S, Burns JS, De Santis G, Loschi P, Pignatti M, Di Benedetto F, Ballarin R, Di Gregorio C, Guarneri V, Piccinini L, Horwitz EM, Paolucci P, Conte P, Dominici M (2011) Understanding tumor-stroma interplays for targeted therapies by armed mesenchymal stromal progenitors: the mesenkillers. Am J Cancer Res 1(6):787–805 (Epub 2011 May 28)
33. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5:309–313
34. Horwitz EM, Gordon PL, Koo WK, Marx JC, Neel MD, McNall RY, Muul L, Hofmann T (2002 Jun 25) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and

stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci USA 99(13):8932–8937
35. Ip JE, Wu Y, Huang J, Zhang L, Pratt RE, Dzau VJ (2007) Mesen- chymal stem cells use integrin beta1 not CXC chemokine receptor 4 for myocardial migration and engraftment. Mol Biol Cell 18:2873–2882
36. Karp JM, Leng Teo GS (2009) Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell 4:206–216
37. Kidd S, Spaeth E, Watson K, Burks J, Lu H, Klopp A, Andreeff M, Marini FC (2012) Origins of the tumor microenvironment: quantitative assessment of adipose-derived and bone marrow-derived stroma. PLoS One 7(2):e30563 (Epub 2012 Feb 20)
38. Kim J, Hematti P (2009) Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. Exp Hematol 37:1445–1453
39. Koç ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM (2000) Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture- expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 18:307–316
40. Koç ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W (2002) Allogeneic mesenchymal stem cell infusion for treatment of metachro- matic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). Bone Marrow Transplant 30:215–222
41. Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen HH, Fritzges D, Izbudak I et al (2005) Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. Circulation 112:1451–1461
42. Lange C, Brunswig-Spickenheier B, Cappallo-Obermann H, Eggert K, Gehling UM, Rudolph C, Schlegelberger B, Cornils K, Zustin J, Spiess AN, Zander AR (2011) Radiation rescue: mesenchymal stromal cells protect from letal irradiation. PLoS One 6(1):e14486
43. Laurila JP, Laatikainen L, Castellone MD, Trivedi P, Heikkila J, Hinkkanen A, Hematti P, Laukkanen MO (2009) Human embryonic stem cell-derived mesenchymal stromal cell transplantation in a rat hind limb injury model. Cytotherapy 11(6):726–737
44. Le Blanc K, Mougiakanos D (2012) Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol 12:383–396
45. Lee JW, Fang X, Gupta N, Serikov V, Matthay MA (2009) Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci USA 106(38):16357–16362 (Epub 2009 Aug 31. (a))
46. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ (2009) Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 5(1):54–63 (b)
47. Maggini J, Mirkin G, Bognanni I, Holmberg J, Piazzón IM, Nepomnaschy I, Costa H, Cañones C, Raiden S, Vermeulen M, Geffner JR (2010) Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS One 5(2):e9252
48. Moll G, Jitschin R, von Bahr L, Rasmusson-Duprez I, Sundberg B, Lönnies L, Elgue G, Nilsson-Ekdahl K, Mougiakakos D, Lambris JD, Ringdén O, Le Blanc K, Nilsson B (2011) Mesenchymal stromal cells engage complement and complement receptor bearing innate effector cells to modulate immune responses. PLoS One 6(7):e21703 (Epub 2011 Jul 1)
49. Moll G, Rasmusson-Duprez I, von Bahr L, Connolly-Andersen AM, Elgue G, Funke L, Hamad OA, Lönnies H, Magnusson PU, Sanchez J, Teramura Y, Nilsson-Ekdahl K, Ringdén O, Korsgren O, Nilsson B, Le Blanc K (2012) Are therapeutic human mesenchymal stromal cells compatible with human blood? Stem Cells 30(7):1565–1574
50. Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM et al (2009) Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 15:42–49

51. Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG (2003) Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci USA 100:8407–8411
52. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG (2007) Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci USA 104(26):11002–11007 (Epub 2007 Jun 14)
53. Parekkadan B, Upadhyay R, Dunham J, Iwamoto Y, Mizoguchi E, Mizoguchi A, Weissleder R, Yarmush ML (2011) Bone marrow stromal cell transplants prevent experimental enterocolitis and require host CD11b + splenocytes. Gastroenterology 140(3):966–975 (Epub 2010 Oct 16)
54. Pessina A, Bonomi A, Coccè V, Invernici G, Navone S, Cavicchini L, Sisto F, Ferrari M, Viganò L, Locatelli A, Ciusani E, Cappelletti G, Cartelli D, Arnaldo C, Parati E, Marfia G, Pallini R, Falchetti ML, Alessandri G (2011) Mesenchymal stromal cells primed with paclitaxel provide a new approach for cancer therapy. PLoS One 6(12):e28321 (Epub 2011 Dec 20)
55. Pittenger MF, Mackay AM, Beck SC, et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
56. Potapova IA, Brink PR, Cohen IS, Doronin SV (2008) Culturing of human mesenchymal stem cells as three-dimensional aggregates induces functional expression of CXCR4 that regulates adhesion to endothelial cells. J Biol Chem 283:13100–13107
57. Ren C, Kumar S, Chanda D, et al (2008) Therapeutic potential of mesenchymal stem cells producing interferon-alpha in a mouse melanoma lung metastasis model. Stem Cells 26:2332–2338
58. Rieger K et al (2005) Mesenchymal stem cells remain of host origin even a long time after allogeneic peripheral blood stem cell or bone marrow transplantation. Exp Hematol 33:605–611
59. Rombouts WJ, Ploemacher RE (2003) Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia 17(1):160–170
60. Ruster B, Gottig S, Ludwig RJ, Bistrian R, Muller S, Seifried E, Gille J, Henschler R (2006) Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. Blood 108:3938–3944
61. Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131:324–336
62. Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, Wohlgemuth R (2008) Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. Nat Med 14:181–187
63. Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP, Wohlgemuth R (2008) Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. Nat Med 14:181–187
64. Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H (2008) Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 180(4):2581–2587
65. Schenk S, Mal N, Finan A, Zhang M, Kiedrowski M, Popovic Z, McCarthy PM, Penn MS (2007) Monocyte chemotactic protein-3 is a myocar-dial MSC homing factor. Stem Cells 25:245–251
66. Schofield R (1983) The stem cell system. Biomed Pharmacother 37:375–380
67. Schraufstatter IU, Discipio RG, Zhao M, Khaldoyanidi SK (2009) C3a and C5a are chemotactic factors for human mesenchymal stem cells, which cause prolonged ERK1/2 phosphorylation. J Immunol 182:3827–3836
68. Segers VFM, Van Riet I, Andries LJ, Lemmens K, Demolder MJ, De Becker AJML, Kockx MM, De Keulenaer GW (2006) Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. Am J Physiol Heart Circ Physiol 290:H1370–H1377
69. Sémont A, Mouiseddine M, François A, Demarquay C, Mathieu N, Chapel A, Saché A, Thierry D, Laloi P, Gourmelon P (2010) Mesenchymal stem cells improve small intestinal

integrity through regulation of endogenous epithelial cell homeostasis. Cell Death Differ 17(6):952–961 (Epub 2009 Dec 18)
70. Shi M, Li J, Liao L, Chen B, Li B, Chen L, Jia H, Zhao RC (2007) Regulation of CXCR4 expression in human mesenchymal stem cells by cyto-kine treatment: role in homing efficiency in NOD/SCID mice. Haematologica 92:897–904
71. Shi C, Jia T, Mendez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, Leiner I, Li MO, Frenette PS, Pamer EG (2011) Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. Immunity 34:590–601
72. Simmons PJ, Przepiorka D, Thomas ED, Torok-Storb B (1987) Host origin of marrow stromal cells following allogeneic bone marrow transplantation. Nature 328:429–432
73. Steingen C, Brenig F, Baumgartner L, Schmidt J, Schmidt A, Bloch W (2008). Characterization of key mechanisms in transmigration and invasion of mesenchymal stem cells. J Mol Cell Cardiol 44(6):1072–1084 (Epub 2008 Mar 19)
74. Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, Umezawa A, Kijima H, Fukuda S, Saijo Y (2011) Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med 17(7–8):579–587 10.2119/molmed.2010.00157. Epub 2011 Mar 11
75. Thankamony SP, Sackstein R (2011) Enforced hematopoietic cell E- and L-selectin ligand (HCELL) expression primes transendothelial migration of human mesenchymal stem cells. Proc Natl Acad Sci USA 108(6):2258–2263 (Epub 2011 Jan 21)
76. Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F (2009) Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics. Circ Res 104(3):398–402 (Epub 2008 Dec 18)
77. von Bahr L, Batasis I, Moll G, et al (2012) Analysis of tissues following mesenchymal stromal cell therapy in human indicates limited long-term engraftment and no ectopic tissue formation. Stem Cells 30:1575–1578
78. Walczak P, Zhang J, Gilad AA, Kedziorek DA, Ruiz-Cabello J, Young RG, Pittenger MF, van Zijl PCM, Huang J, Bulte JWM (2008) Dual-modality monitoring of targeted intraarterial delivery of mesenchymal stem cells after transient ischemia. Stroke 39:1569–1574
79. Wang Y, Zhang D, Ashraf M, Zhao T, Huang W, Ashraf A, Balasubramaniam A (2010) Combining neuropeptide Y and mesenchymal stem cells reverses remodeling after myocardial infarction. Am J Physiol Heart Circ Physiol 298(1):H275–H286 (Epub 2009 Nov 6)
80. Wei L, Fraser JL, Lu ZY, Hu X, Yu SP (2012) Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. Neurobiol Dis 46(3):635–645 (Epub 2012 Mar 9)
81. Wu J, Sun Z, Sun H-S, Wu J, Weisel RD, Keating A, Li Z-H, Feng Z-P, Li R-K (2008) Intravenously administered bone marrow cells migrate to damaged brain tissue and improve neural fnction in ichemic rats. Cell Transplant 16:993–1005
82. Xu S, Menu E, De Becker A, Van Camp B, Vanderkerken K, Van Riet I (2012) Bone marrow-derived mesenchymal stromal cells are attracted by multiple myeloma cell-produced chemokine CCL25 and favor myeloma cell growth in vitro and in vivo. Stem Cells 30(2):266–279
83. Yilmaz G, Vital S, Yilmaz CE, Stokes KY, Alexander JS, Granger DN (2011) Selectin-mediated recruitment of bone marrow stromal cells in the postischemic cerebral microvasculature. Stroke 42(3):806–811 (Epub 2011 Jan 21)
84. Zhang M, Mal N, Kiedrowski M, Chacko M, Askari AT, Popovic ZB, Koc ON, Penn MS (2007) SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. FASEB J 21:3197–3207