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Mesenchymal stem cells and their microenvironment

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TABLE OF CONTENTS

1. ABSTRACT

Mesenchymal stem cells (MSC) are multipotent stem cells that hold promise for an expanding list of therapeutic uses, not only due to their ability to differentiate into all connective tissues including bone, fat and cartilage, but additionally due to their trophic and anti-inflammatory effects which contribute to healing and tissue regeneration. Ongoing research is starting to illuminate important aspects of the microenvironmental niche, which supports MSC self-renewal. In this review, we summarize recent findings on cellular structures and molecular pathways that are involved in regulation of MSC self-renewal versus differentiation, and in retention of MSCs within the niche versus mobilization and recruitment to sites of injury. In addition, the contribution of MSCs to the structure and function of hematopoietic and cancerous niches is discussed.

2. DEFINITION OF MESENCHYMAL STEM CELLS (MSC)

MSCs are stromal-like cells that are characterized by a $CD105^+/CD73^+/CD90^+/CD45^-/CD34^-/CD11b^-/CD19^-/HLA\text{-}DR^-$ cell surface signature (1); they are capable to differentiate into all connective cell types and tissues including bone, cartilage, muscle, ligament, tendon, stromal fibroblasts and adipocytes. MSCs contribute to the regeneration of injured tissue by this cell replacement mechanism and by the ability to secrete trophic factors (for reviews see: (2, 3)).

The minimal set of standard conditions which defines MSCs by their cell surface signature as described above, their adherent nature and their ability to differentiate to osteoblasts, adipocytes and chondroblasts

in vitro, was established in 2006 (1) and has allowed a more uniform characterization of MSC. However, this definition stops short from fulfilling the criteria for describing a stem cell in the strict sense, i.e. the property that an individual stem cell is capable of self-renewal. It has been difficult to show the ability of MSC for self renewal and the clonality of MSCs, but this has been achieved recently (4-7), indicating the true stem cell nature of these cells, although it is safe to assume that MSC cultures consist of a mixture of bona fide stem cells and a much larger pool of progenitors (8), which cannot be distinguished predictively from each other with our current knowledge.

Originally MSC were isolated from bone marrow as rare cells in the mononuclear cell fraction that adhere to tissue culture plastic (9). Since these cells do not express any unique, recognized cell surface marker which is specific only for MSC, this simple approach allowed much progress to be made, but by the same token this approach is undoubtedly one of the reasons for often contradictory results in this field of research, - and there is no doubt that different laboratories have isolated and characterized different cell populations. Prospective isolation procedures for MSC are still in the process of being optimized and standardized, but should eventually lead to a better defined cell population. As more standardization has been achieved lately by fulfillment of the minimal criteria described above, new complexity has arisen because MSC have been isolated from practically any tissue including bone marrow, trabecular bone, fat, synovium, vascular wall, dental pulp, muscle, kidney, lung, brain, pancreas, stomach, umbilical cord and placenta (5, 10-15).

While MSC hold great promise for tissue repair, better characterization of the precise properties and nature of MSC is necessary to realize the full clinical potential of these cells. Their capacity to form various connective tissues is only one of the possible clinical applications of these cells. In many disease models MSC participate in tissue repair without differentiation into any specific mesenchymal lineage, but largely due to the production of numerous growth factors - such as bFGF, IGF-1, HGF, BMP1, TGF-beta and SCF - and angiogenic factors including VEGF. These factors inhibit ischemia induced apoptosis and contribute trophic support in the damaged tissue (16, 17). Furthermore the immune-suppressive functions of MSCs, which include the down-regulation of pro-inflammatory cytokines, T cell suppression and inhibition of NK cell and dendritic cell functions, contribute to the switch from a state of inflammation to a state of healing (18-20). For these reasons MSCs represent a promising new therapeutic approach in inflammatory, ischemic and auto-immune diseases, where recent clinical trial results look promising (21-25). However, there have also been failed clinical trials, sometimes due to poor study design, but also because the basic biology of MSCs is not adequately understood. The current rush to the clinic has at times dissipated limited resources on premature clinical trials, when more patience and a better understanding of the basic biology could have resulted in more informed and successful therapeutic interventions in the long run. In particular, very little is known about the local microenvironment under which MSCs maintain their stemness, function optimally and which allows them to retain their repair capacity throughout life. This microenvironment may not always be optimal leading to a gradual loss of MSCs over a life-time, and indeed it has been described that the frequency of MSC decreases with increased age (17, 26), perhaps one of the reasons for delayed recovery from tissue injury in old age.

Insufficient knowledge about an optimal microenvironment for MSC also poses a problem during *in vitro* expansion of MSCs. While these cells can be easily cultured and extensively expanded, they gradually lose their potential to differentiate into all of the mesenchymal lineages over a number of passages and eventually become senescent, because the culture environment only partially replicates natural niche conditions. From all of the above it is clear that it is essential to obtain a better understanding of the niche for MSCs.

3. DEFINITION OF THE STEM CELL NICHE

The term “stem cell niche”, which was originally proposed by Schofield for the hematopoietic stem cell niche (27) refers to the microenvironment in which stem cells reside. This microenvironment consists of different cell types, ECM molecules and soluble factors regulating stem cell activities, and plays an important role for the self-renewal of stem cells, which will begin to differentiate outside this niche. The balance between self-renewal and differentiation is dependent on this stem cell niche.

4. WHAT IS THE STEM CELL NICHE FOR MSC?

As mentioned above MSC have been isolated from nearly any tissue in the body, and given the differences in microenvironment of various tissues, it is astounding that they retain their stem cell potential to differentiate into the various types of connective tissue under external influences that cannot be identical. So what milieu is shared in these different locations which can provide a similar MSC niche in all these surroundings? There is increasing evidence that MSC exist in a perivascular location and share a number of cell surface markers with pericytes (5, 28-32). This does not mean that pericytes are MSC, but it suggests that pericytes are derived from MSCs. Such a perivascular location of MSCs would make a lot of sense for a cell type that is constantly recruited to areas of tissue injury, - although it does not exclude the possibility that there may be additional niches. A perivascular location seems ideal positioning for cells that contribute to tissue repair through the production of a variety of growth factors that work in a paracrine fashion. Such a perivascular location would also guarantee a similar niche for MSC in different organs, such that MSC self-renewal is enabled throughout the body. Nevertheless, the conditions would

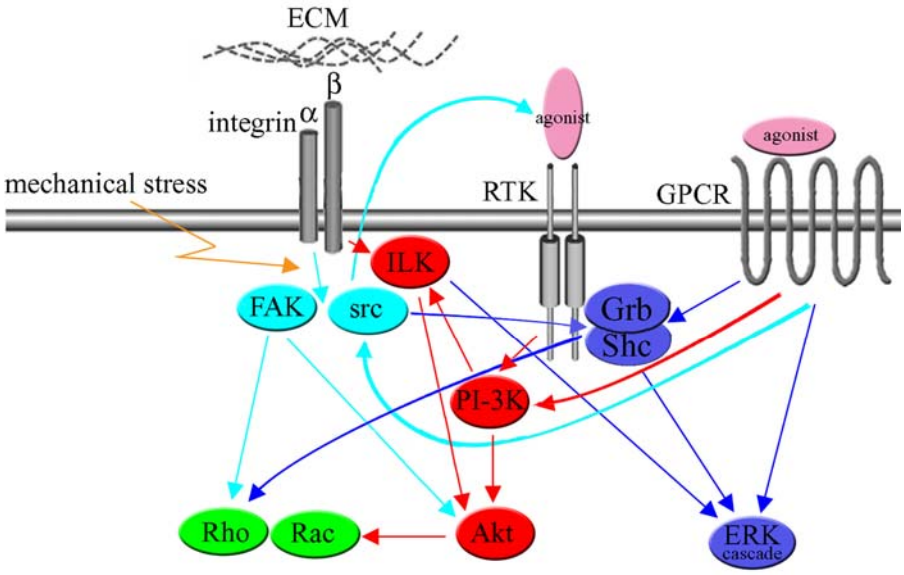

Figure 1. Simplified depiction of the cross-talk between different signaling pathways important for MSC survival and self-renewal. Integrin-mediated adhesion of MSCs to the ECM is necessary for sustained MSC survival. Outside-in integrin signaling leads to FAK and Src activation, which in turn result in activation of the Akt pathway and the ERK cascade, which provide survival and proliferation signals. It also leads to modulation of the small GTPases Rac, Rho and cdc42, which regulate cell motility. Following agonist stimulation, receptor tyrosine kinase receptors (RTKs) such as the EGF receptor and the FGF receptors dimerize and are autophosphorylated, which leads to the assembly of adapter molecules including Grb2 and Shc that serve as a scaffold for the assembly of downstream signaling molecules ultimately resulting in activation of the ERK and PI-3K/Akt cascade as well as in that of the small G-proteins Rac and Rho. There are additional activation pathways induced by RTK activation such as the JAK/Stat pathway, which has been omitted in the depiction. Activation of G-protein coupled receptors (GPCRs) - for instance by chemotactic factors - similarly leads to activation of ERK and PI-3K pathways. Although there are some variations depending on the specific G-protein intermediate, ERK and Akt activation are seen with all classes of heterotrimeric G-proteins. Many GPCR agonists can also transactivate RTKs, e.g. by metalloprotease-mediated cleavage of membrane-anchored pro-growth factors, which in turn activate their specific receptor. Finally mechanical stress can activate the same signaling cascades in an integrin and GPCR-dependent fashion.

not be identical, since there are subtle differences between the endothelial cells of various vascular beds. Indeed it has been noted that MSC co-cultured with macrovascular endothelial cells acquire smooth muscle cell markers, while co-cultures with microvascular endothelial cells do not (33), which is consistent with the presence of myofibroblasts in the wall of large, but not of small vessels. Furthermore depending on the tissue of origin, MSC show a greater propensity to differentiate to cartilage and bone, if they are bone marrow derived and to differentiate to fat, if isolated from adipose tissue (34). Adipose tissue derived MSC also seem enriched for immune response genes (35). There are many additional examples that indicate the tendency of MSC of a specific tissue to preferentially differentiate into the type of connective tissue that is needed in a specific location, and local MSCs may serve as the first line of MSCs that contribute to tissue repair prior to MSC mobilization from more distant sites. Such a recruitment mechanism of local MSCs has been suggested for instance for MSCs in the renal capsule following ischemic kidney injury (36).

Specific sites for the MSC niche have been suggested for some tissues. For instance it has been proposed that the connective sheath and the papilla of the hair follicle represent the anatomical niche for cutaneous MSC (37), and trabecular bone appears to be a site in which bone marrow MSCs are enriched (38, 39). It should be pointed out, however that these additional sites may not differ from the perivascular niche, which was described above, since capillary networks are universally present. Since a major function of the stem cell niche is to provide an optimal environment for the self-renewal of these cells, it is important to consider the parameters that play a role in the self-renewal of MSCs next.

5. CONDITIONS THAT SUPPORT MSC SELF-RENEWAL VERSUS DIFFERENTIATION

In order to self renew, stem cells need to be protected from differentiation signals and from apoptosis, and the niche provides the adhesion molecules, soluble factors and conditions that allow this in a concerted fashion. These soluble factors and ECM components activate various kinase cascades including the ERK1/2 MAPK and the PI-3K pathways – as shown in schematic form in Figure 1. These pathways appear to be important for MSC self renewal, but the exact

conditions have not been defined yet. The strength, kinetics and cellular location of activation of these pathways all play a role as well as the temporal coordination between several pathways. For example EGF, which stimulates the EGFR1 on MSCs, strongly activates the MAP kinases ERK1/2 and Jnk1, the Stat3 and PKC pathways and weakly stimulates the PI-3K pathway, and high concentrations of EGF induce osteogenic differentiation in MSCs (40). When the ERK1/2 pathway was inhibited under these conditions, adipogenic differentiation resulted instead (40). EGF and PDGF activate similar downstream signaling pathways, except that PDGF induces a stronger activation of the PI-3K pathway, - but PDGF does not induce osteogenic differentiation of MSC (41). However, in the presence of a PI-3K inhibitor, PDGF causes osteogenic differentiation (41), suggesting that concomitant PI-3K stimulation prevents ERK1/2-dependent osteogenic differentiation. There are even examples, where the same growth factor can produce contradictory results: for instance, high concentrations of covalently tethered EGF, which restrict signaling to the cell surface, result in increased osteogenic differentiation of MSCs, while low concentrations of soluble EGF, which induce receptor internalization, are anti-osteogenic (42). A better understanding of the signaling mechanisms that retain MSCs in their undifferentiated state should help to improve the quality of *in vitro* expanded MSCs in the future.

A number of cytokines and growth factors including bFGF, LIF, EGF/HB-EGF, HGF, PDGF, IL-6 and Wnt (3a) have been implicated in MSC self-renewal (43-50) because of their demonstrated role in the self-renewal of other stem cell types. Although self-renewal has not been tested in most cases where MSCs were stimulated with these factors, - mostly due to lack of an appropriate self-renewal assay for MSCs -, there is reasonable evidence that some of these factors inhibit osteogenic and adipogenic differentiation, but increase proliferation, which implies that they might support self-renewal. This is for instance the case for bFGF (43), HB-EGF (44), Wnt (3a) (46, 47) and HGF (48), which all activate multiple signaling pathways including MAP kinases, the PI-3K cascade, Smads, Stat3, calcium flux and small G proteins of the rho family. Such a plethora of activation pathways makes it difficult to understand the molecular determinants involved in MSC self renewal. It appears, however, that there is a coordinated activation – or inhibition - of several pathways that might be involved in the self renewal of MSCs in analogy with human embryonic stem cells (ESCs) in which both bFGF and activin/TGF-beta activation pathways are necessary to prevent differentiation. Indeed, the addition of a mixture of bFGF, TGF-beta and PDGF was sufficient to culture MSC for 5 passages in the absence of FCS (49), while retaining the potential of these cells to differentiate into osteoblasts, adipocytes and chondrocytes. This indicates again that the balanced action of several factors and their downstream signaling cascades is necessary to maintain undifferentiated MSCs. Also in analogy to ESCs some of these pathways inhibit differentiation into a specific progenitor, for instance HGF inhibits BMP-induced nuclear translocation of activated Smad 1, 5 and 8 thus preventing MSC differentiation to osteoblasts (48).

The addition of bFGF to MSC cultures was more effective in supporting self-renewal in low density cultures (43), which suggests that soluble factors produced by MSCs contribute to differentiation and/or senescence of MSC if they are present in too high concentrations. It was recognized several years ago that propagation of MSC in low density cultures allows increased expansion (51), and several genes linked to cell motility are up-regulated in low density cultures (52). Such a density-dependent behavior appears useful, since MSC are rare cells *in vivo*, - but when they accumulate in an area of tissue injury, it would be beneficial for them to differentiate into the appropriate tissue rather than to self renew.

Some factors that are produced by MSC are known to induce differentiation into a specific mesenchymal lineage. These factors include several BMPs (53, 54) and bFGF (49), which both play a role in osteogenic differentiation, and TGF-β, which is necessary for chondrogenic differentiation (49).

Many of the angiogenic and trophic factors produced by MSCs support cells of the surrounding tissue as well as MSCs themselves: MSCs can produce VEGF, bFGF, PDGF, angiopoietin, CXCL8/IL-8 and other angiogenic factors (55), which all play a role in vasculogenesis, but MSC also respond to most of these factors themselves (56, 57), although VEGF appears to signal through the PDGFR in these cells (58). In fact such angiogenic and trophic effects on the surrounding cells appear to be responsible for much of the therapeutic effect of MSCs (16, 128-130). In addition many of the growth factors produced by MSC including bFGF, VEGF, PDGF, and TGF-beta are also chemotactic factors for these cells.

MSCs may contribute significantly to their microenvironment through their own gene expression profile and through the production of factors that function in an autocrine or paracrine fashion, which would make them less dependent on a niche created by other cell types. Indeed MSC have been reported to express a large number of genes characteristic of stem cells in general even including pluripotency markers. There have been reports that they express Oct4, Nanog, Sox2, SSEA3, SSEA4, Rex1, c-myc, nucleostemin, Nodal, Sca1, Snail2, and others (59-66), although there is controversy about the expression of some of these factors. To some degree these contradictory findings may be due to differences in culture conditions such as cell origin, isolation method, passage number and media. For instance, Sca1 is expressed by murine, but not by human MSC (62). Although this pathway does not apply to humans, it is interesting in the mouse system, because aged Sca-/- mice develop osteoporosis due to decreased self-renewal of MSCs (62). This example also illustrates

that one has to be cautious about generalizing the lessons learned from mouse cells. As with ESCs, there appear to be important differences between the self-renewal and differentiation pathways of rodent and human MSC. For instance, BMPs play a central role in the osteogenic differentiation of rodent MSC (67-69). In contrast in human MSCs BMPs are insufficient to induce an osteogenic response, although these cells express functional BMP receptors (70). Similarly, classical Wnt signaling is essential for osteogenic differentiation in the mouse (71), but may have contrary effects in human MSCs (46). Specifically in the case of Oct4 caution is warranted for both species, however. The Oct4 gene is irreversibly silenced in somatic tissues, which makes it difficult to envision its reactivation in untransformed adult stem cells, and in several reports no precautions were taken to exclude false positive results including pseudogene expression (72). Nevertheless, this still leaves a plethora of stem cell genes in MSC that must be controlled in a way that will not lead to cancerous transformation. It is far from clear, how this is achieved, but MSC have been found safe in clinical applications (21, 25) and their delivery is not associated with tumor formation as is seen with ESC and induced pluripotent stem cells. Replicative senescence is certainly one mechanism by which this can be achieved, and replicative senescence occurs both during *in vitro* MSC expansion as well as over the life time of an individual (73), a fact which has to be taken into account, when autologous MSC transplantation is considered in elderly patients.

6. THE EFFECT OF THE EXTRACELLULAR MATRIX (ECM) ON MSC

The extracellular matrix including the growth factors and cytokines that associate with it, are an important component of the stem cell niche, and the survival of MSCs depends on their ability to adher to the ECM. When expanded *in vitro*, MSC gradually lose their ability to differentiate into the various mesenchymal cell types over a number of passages. However, when cultured on a basement membrane-like ECM, MSC retained their osteogenic, chondrogenic and adipogenic potential for a prolonged period of time compared to cells grown on tissue culture plastic (74). Similarly, MSC cultured on denatured collagen type I showed continued adipogenic and osteogenic differentiation potential (75), while culture on laminin-5 induced osteogenic differentiation of MSC even in the absence of specifically osteogenic medium (76).

This effect of the ECM composition may be partially explained due to sequestration of endogenously produced growth factors, but the physical property of the substratum also plays an important role. For instance varying the elasticity of the matrix influenced MSC differentiation: rigid matrices – resembling the dense structure of bone - favored expression of osteogenic genes, while softer matrices – similar to the elasticity of striated muscle – induced muscle-specific genes (77). Substrate elasticity also had a major influence on the production of various soluble factors by MSC, with concentrations of IL-8 varying up to 90-fold depending on the rigidity of the substrate (78). Similarly, when chondrogenesis was assessed in cross-linked methacrylated hyaluronic acid hydrogels, the macromer density influenced MSC chondrogenesis: high density macromers resulted in increased chondrogenesis, but of inferior quality than seen with lower density gels (79).

Mechanical strain is a further factor that influences MSCs: it improves osteogenic differentiation (80), and bone-like ECM synthesized *in vitro* is able to enhance osteoblastic differentiation of MSC in particular when combined with fluid shear stress exposure even in the absence of osteogenic medium (81).

Since integrins function as adhesion receptors for ECM proteins, one would expect that their expression levels will influence the behavior of MSCs. Indeed early MSC progenitors, defined by their smaller size and expression of podocalyxin-like protein (PODXL), selectively express $alpha_4$ and $alpha_6$ integrins, which are lost during culture (82). Freshly isolated MSC do not express the vitronectin receptor $alpha_v$ $beta_5$, but express low levels of the fibronectin receptor $alpha_5beta_1$ and the collagen receptors $alpha_1beta_1$, $alpha_2beta_1$ and $alpha_3beta_1$ (83). Upon culture $alpha_vbeta_5$ is up-regulated. Furthermore, increased expression of $alpha_vbeta_5$, and of $alpha_5beta_1$ is observed during chondrogenic differentiation (83), and up-regulation of $alpha_2beta_1$ (84) and $alpha_5beta_1$ (85) is necessary for osteogenic differentiation, while $alpha_5beta_1$ expression is down-regulated during adipogenic differentiation (86).

Since one of the major signaling pathways of integrins activates FAK and downstream of it the Rho family of GTPases, it would be expected that this pathway may have effects on MSC biology, and recent results indicate that prolonged activation of this integrin signaling pathway supports osteogenic differentiation. It has long been known that the plating density affects osteogenic and adipogenic differentiation of MSC with preferential adipogenesis at high cell densities and improved osteogenesis at low cell densities (87), - a condition that favors increased Rho activation. When a micropatterning technique was used to control MSC cell shape and the degree of cell spreading, it was found that spread cells underwent osteogenesis, and unspread, round cells became adipocytes (88). Osteogenesis depended on RhoA/ROCK activity generating actin-myosin tension as present in spread-out cells such that expression of constitutively active RhoA resulted in osteogenesis, while dominant negative RhoA expression caused adipogenesis (88). Activation of FAK, part of the same integrin activation pathway was also necessary for osteogenic differentiation (89).

Modulation of integrin signaling pathways similarly plays a role in adipogenic differentiation. Overexpression of α5 integrin in preadipocytes

attenuated differentiation and caused increased Rac activity, and constitutively active Rac inhibited adipogenesis (86).

From all this it is clear that cues from the ECM of a specific mesenchymal tissue play an essential role in the tissue-specific differentiation of MSCs, and although understanding of the basic biology involved in MSC differentiation still has many gaps, there is great interest and much active research in the development of three-dimensional matrices and unconventional culturing methods to optimize MSCs for tissue repair purposes.

7. MSC AND TISSUE DAMAGE

MSCs serve as a pool of cells that is recruited to damaged tissue, whenever there is tissue injury. While MSCs do not seem to circulate in the vasculature under physiological conditions, it seems likely that they are released into the vasculature, when there is increased demand for these cells during any kind of tissue injury (90). But although MSC accumulate in damaged tissue to some degree (91), recruitment of circulating MSC is very inefficient, when compared to leukocyte extravasation (92). Furthermore, following i.v. delivery, MSC tend to lodge in the lungs, especially in mice. Although this appears to be at least partially because these cells are arrested in the primary vascular bed due to the small diameter of the murine microvasculature, - delivery of circulating MSC to damaged tissues is generally poor and better tissue delivery would greatly improve the tissue repair capacity of these cells.

MSC are cells that are relatively resistant to injury by hypoxia (93, 94), irradiation (95, 96) and chemotherapy (97), which is not surprising for a cell type which is destined to repair tissue damage, and therefore has to survive under adverse conditions. MSCs seem to be resistant in general to certain apoptotic pathways perhaps due to very low expression of caspase 8 and caspase 9 (98). In fact protein fragments for instance of EGF released from apoptotic endothelial cells, protect MSCs from apoptosis (99). While all of this documents that MSCs are capable to survive under adverse conditions, it is not clear, whether MSCs treated in such a way are still capable of self renewal. This issue has not been addressed in any of the reports showing the resistance to cell death in these cells. There clearly is a decline in the numbers of MSCs over a life-time (100), - and MSCs derived from older individuals are capable of fewer population doublings *in vitro* than those obtained from younger subjects (101), indicating that there is a limit to the regenerative potential of these cells.

It seems highly likely that there is a similar niche retention/mobilization equilibrium going on with MSC as there is with HSC, except that the molecular players involved in this process are not yet defined in the case of MSCs. It will be important to identify which signals produced by injured tissue are sensed by MSCs that allow the release of MSCs from their niche, i.e. cause mobilization of MSCs, which subsequently allows their recruitment to the site of injury.

In the absence of an inflammatory reaction, MSC do not circulate, and there remains some controversy, to what extent they can be released to the circulation, when there is tissue injury (102). It is not entirely clear what proportion of MSC attracted to an area of tissue damage is recruited from the circulation versus the surrounding tissue stroma, but in either scenario, MSC can be recruited by stimuli generated in injured tissues.

Figure 2 summarizes how inflammatory mediators recruit first leukocytes, then MSCs to an area of tissue injury. Tissue damage causes up-regulation/activation of endothelial cell adhesion molecules, the production of various chemokines and activation of the complement cascade leading to the production of C3a and C5a. Following leukocyte adhesion to the endothelium, these chemotactic factors cause the migration of leukocytes, - initially of neutrophils - across the endothelial barrier. This is later followed by the influx of monocytes. Various chemokines are released by the infiltrating neutrophils and monocyte/macrophages augmenting the inflammatory response. In particular, macrophages produce high concentrations of chemokines and of various growth factors including bFGF, VEGF, PDGF and TGF-beta. Various proteases derived from neutrophils, macrophages and endothelial cells further enhance the availability and activity of some of these factors for instance by releasing VEGF bound to the extracellular matrix and activating latent TGF-beta. A large number of factors produced under these circumstances show chemotactic activity for MSCs including bFGF (103), VEGF (104), PDGF (105), TGF-beta (106), C3a (107), C5a (107), sphingosine-1-phosphate (108), and various chemokines (for a review see (102)). While it is clear that MSC are recruited by chemokines, there is a lot of confusion currently about specific chemokines - with contradictory results coming from different laboratories (102, 109-116), which may at least partially be explained by different culture conditions. Especially, the role of SDF-1alpha/CXCL12 is controversial in this respect. For instance, we find no chemotactic response towards SDF-1alpha and do not see any cell surface expression of its receptor, CXCR4 - in spite of detectable CXCR4 message by RT-PCR, - and most laboratories see cell surface CXCR4 expression at most on a very small fraction of cultured MSCs. However, MSCs produce SDF-1alpha constitutively, and it is possible that this results in desensitization and internalization of CXCR4 in an autocrine fashion, as intracellular, but not cell surface expression of CXCR4 and other chemokine receptors has been described (117). This may also be the case for IL-8/CXCL8 and MIP-1alpha /CCL3 for which there exist similarly disparate findings with some reports observing chemotaxis, while others do not. Both IL-8 and MIP-1α can be produced by MSC in concentrations that may be high enough to cause receptor desensitization. In all cases, however, cell surface expression of chemokine receptors was only seen in a fraction of cells ranging from 2% to 70% with large differences described by different laboratories. In

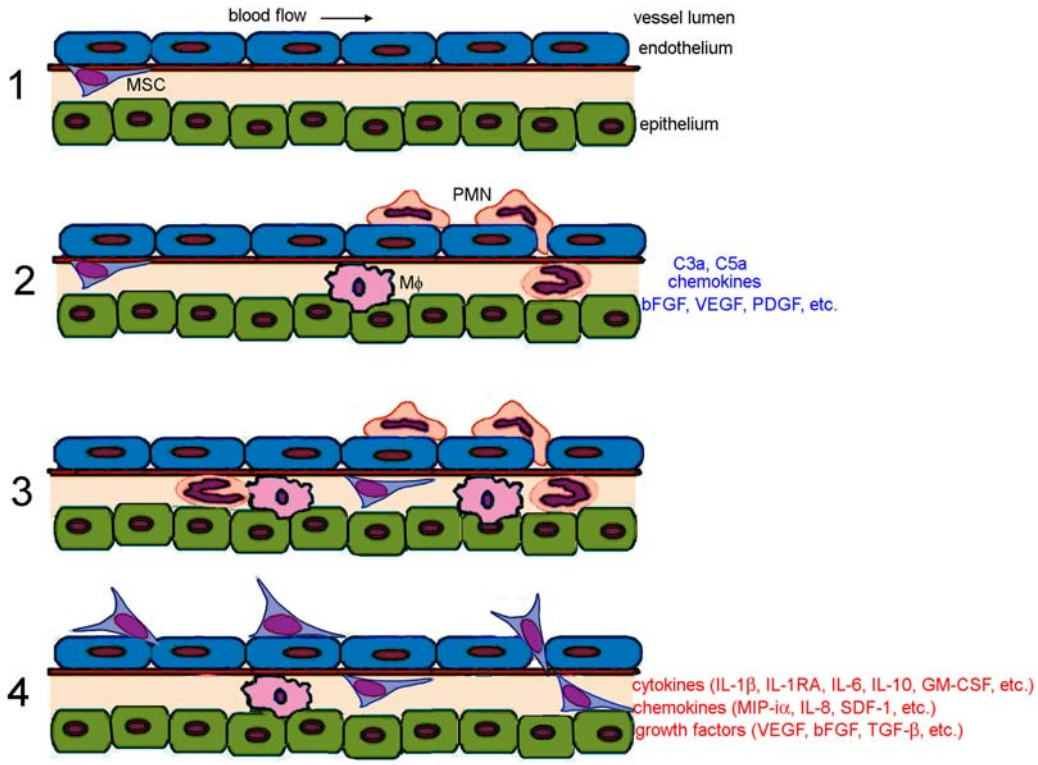

Figure 2. MSC recruitment to an area of tissue injury: Under normal physiological conditions MSCs reside in all tissues, often in a perivascular location (panel 1). Following tissue injury, adhesion molecules on endothelial cells are up-regulated, various chemokines and cytokines are produced and complement is activated leading to the production of C3a and C5a. In the first line of defense circulating neutrophils are chemo-attracted by these factors, primarily by chemokines such as IL-8 and by C5a, followed by monocytes in the second line of defense (panel 2). The attracted leukocytes release proteases, oxidants and various growth factors and chemokines, many of which are chemo-attractants for MSCs. Factors produced during tissue injury that recruit MSCs are shown in blue (panel 2). MSCs can be attracted by two routes: 1) directly from the surrounding interstitial space as depicted for one MSC, which is attracted from the left in panel 2 to the middle in panel 3, or 2) from the circulation through diapedesis through the endothelium (panel 4). MSCs produce a large number of trophic factors and anti-inflammatory factors shown in red that contribute to the resolution of inflammation and the healing process.

contrast all MSC express cell surface receptors for C3a and C5a (107), suggesting that these anaphylatoxins may play an important role in the recruitment of MSC under pathological conditions.

Loss of MSC niche retention mechanisms and subsequent MSC recruitment is expected to follow two routes: 1) recruitment of perivascular MSC from the nearby environment and 2) transendothelial migration of circulating MSC reminiscent of leukocyte diapedesis (see Figure 2). Recruitment of perivascular MSC appears relatively straightforward, since MSC express chemokine receptors and other receptors involved in cell migration, adhesion molecules including VLA-4 and VLA-5, - although at a lower expression level than seen in leukocytes (92) - , as well as MT-MMPs (118) and MMPs, in particular MMP2 (108, 119) that allow invasion of the surrounding matrix.

Much less is known about the mechanisms that allow endothelial adhesion and transmigration of circulating MSCs. In most leukocytes the first step in this process consists of rolling mediated by the interaction of L-selectin and L-selectin receptors such as PSGL-1 and sialyl Lewis^x with endothelial selectins. It is not clear, how rolling proceeds in MSCs, since they express no L-selectin, PSGL-1 and sialyl Lewis^x (117), although it is possible that they express an alternate saccharide, which is able to bind P-selectin (112). Limited rolling could be one of the reasons for the rather poor efficiency with which circulating MSCs are recruited by injured tissues, and engineering MSC to express such fucosylated selectin receptors seems to increase the ability of MSCs to adhere to selectins (92, 120). Such engineering may be used to direct MSC into specific locations: For instance specialized bone marrow vessels express E-selectin constitutively, and following *ex vivo* glycan engineering of these cells, intravenously infused MSC infiltrated the bone marrow much more efficiently than control MSCs (92).

The next step in the extravasation process, firm adherence to the endothelium is similarly not well defined in MSC, although these cells express moderate

levels of VLA-4, the ligand for endothelial VCAM, which is up-regulated in the presence of inflammatory cytokines including TNF-alpha and IL-4. Possible adhesion mediators are the variety of integrins expressed by MSCs as well as CD44, which is highly expressed by MSCs and which binds endothelial hyaluronic acid, a mechanism that has been previously described to participate in lymphocyte extravasation (121), and which has been shown recently to play a role in MSC extravasation (92).

The third step, transmigration, is relatively well understood *in vitro*, where a plethora of chemotactic factors are chemotactic for MSCs as described above. However, the relative importance of various chemo-attractants in the recruitment of MSCs *in vivo* is also not well understood. The complement split products C3a and C5a are interesting in this respect: C3a is produced by all three complement activation pathways (classical, alternative and lectin) and it is generated under all conditions of inflammation or ischemic tissue injury as well as in cancer. But while C3a receptors are expressed by various leukocyte populations, C3a is a weak inflammatory stimulus for two reasons: 1) C3a activates leukocytes only in a weak and transient fashion – even *in vitro* – and does not induce any leukocyte recruitment *in vivo* (122). This is in contrast to the prolonged and potent effect of C3a on MSCs (107), which so far has only been addressed *in vitro*. 2) C3a is inactivated by carboxypeptidase N (123) in plasma suggesting that C3a can only function as a chemo-attractant within the interstitial space *in vivo*. This could be a useful means of directing MSCs to a site of tissue injury by two means, 1) the recruitment of local MSCs within the parenchyma of any injured tissue, and 2) the recruitment of MSCs that have arrested on the endothelium - due to chemo-attraction by other mediators such as chemokines or C5a – from the vascular to the interstitial space. There is evidence that MSC which are injected i.v. are recruited to vascular beds in areas of tissue injury, but in contrast to leukocytes they do not transmigrate to the interstitium immediately, but seem to lodge on the luminal side of the microvasculature for some time, before they finally appear in the interstitium (124, 125). The molecular mechanisms of this process are still unknown, - and there are probably various possibilities, but C3a appears to be ideally suited in this scenario, which could attract MSCs that have adhered to the endothelium, but not leukocytes. Such a mechanism could be instrumental in converting an inflammatory reaction to one of healing.

Sphingosine-1-phosphate, which is released by activated platelets and a strong chemo-attractant for MSCs is also interesting in this context: S1P1 receptor knockout mice were embryonically lethal due to a lack of vascular maturation caused by a deficiency of – MSC-derived – vascular smooth muscle cells and pericytes (126), and indeed sphingosine-1-phosphate induces differentiation of MSC to smooth muscle cells (127) suggesting a mechanism by which thrombus formation – leading to S1P1 production - may change the microenvironment of MSCs such that these cells can contribute to reparative revascularization.

8. CONTRIBUTION OF MSCS TO THE HSC NICHE

Much about what we know about stem cell niches was pioneered for the hematopoietic stem cell (HSC) niche. The osteoblastic and endothelial compartments of the hematopoietic niche, as well as the neurotransmitter component, are discussed in great details in other reviews published in this issue. However it should be remembered, that there are no defined boundaries in bone marrow and that cells from different compartments of the hematopoietic microenvironment are located in close proximity to each other composing an integrated complex regulatory structure.

MSCs contribute to the complex structure of the hematopoietic niche by two different means: 1) MSCs differentiate into osteoblasts whose role in HSC maintenance is well established (131); and 2) MSCs themselves can support HSC proliferation and self-renewal. The latter will be discussed here in greater detail.

In vitro studies demonstrated that MSCs induce proliferation and self-renewal of HSC by a beta-1-integrin-dependent mechanism (132). In addition to improving conditions for HSC expansion, MSCs inhibit their differentiation and apoptosis (133). These *in vitro* findings were also supported by *in vivo* studies, which demonstrated that HSCs transplanted with MSCs engrafted more efficiently than HSCs alone (134). However, another group found that naive MSCs lack a stimulatory effect on HSC self-renewal in-vivo and that co-transplanted MSCs must be beta-catenin-activated (135).

In addition, MSC serve as an abundant source of growth factors for a wide range of hematopoietic processes, which include GM-CSF, SCF, IL-1beta, IL-6, LIF and CXCL12/SDF-1 (136, 137). Direct involvement of MSCs in regulation of HSC fate is supported by gene screening studies which identified a set of genes expressed in MSCs specifically involved in the HSC niche including galectin-1, fibronectin-1, osteopontin, CXCL12, thrombospondin-1 and -2, TGF-beta 2, Angiopoietin-1, ILGFBP-4, FGF-7, SFRP-1 and -2, VCAM-1, and BMPR type 1a (138). The ability to produce Angiopoietin-1 (a pivotal molecule of the HSC "niche" involved in vascular remodeling) was also reported for sub-endothelial CD146+ MSCs, which are capable of transferring a hematopoietic microenvironment to heterotopic sites upon transplantation *in vivo* (139).

MSCs do not only regulate the fate of HSCs, but there is cross-talk between HSCs and MSCs in which HSCs can regulate choices of MSCs. Specifically, HSC contribute adaptively to the niche in an autocrine fashion: Following stress, HSC-derived bone

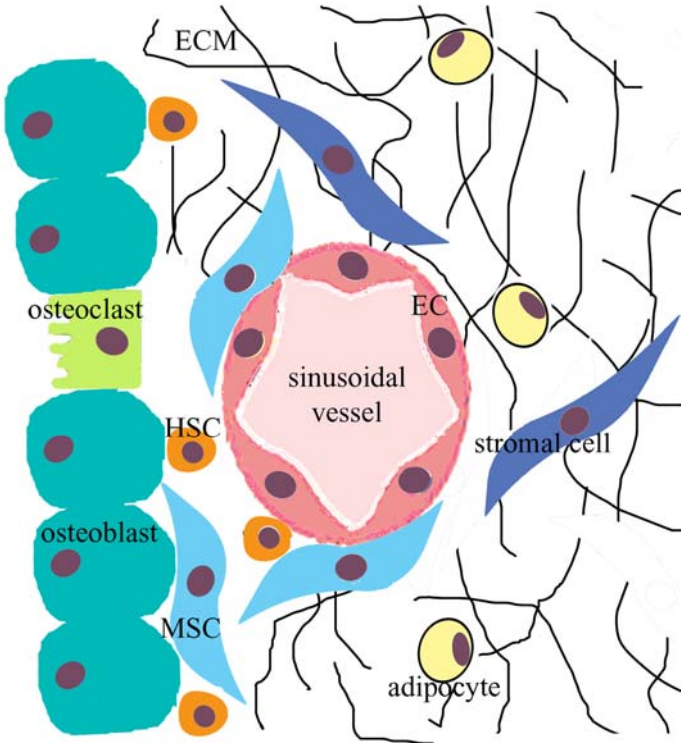

Figure 3. Niches for HSCs and MSCs in bone marrow depicting the symbiotic relationship between HSC and MSC: Throughout ontogeny there is a close association between HSC and MSC (142). In the developed organism, HSC reside in their niche that is formed of osteoblasts as well as the sinusoidal vessel wall, which are also locations enriched for MSCs (5, 171). The stromal components of bone marrow (stromal cells, osteoblasts and adipocytes) are all derived from MSC, while HSC give rise to all hematopoietic cells including monocyte-derived osteoclasts. Blood cells are densely packed in bone marrow, but are omitted from the depiction. Interaction between receptor ligand pairs expressed by HSC and osteoblasts include CXCR4/SDF-1, VLA-4/VCAM, CD44/HA, LFA-1/ICAM and others, but SDF-1, VCAM, CD44, HA and ICAM are similarly expressed by MSCs and endothelial cells (EC).

morphogenic protein 2 (BMP-2) and BMP-6 improve osteogenic differentiation of MSC (140).

The symbiotic relationship between HSC and bone marrow MSC is illustrated in Figure 3. The close association between HSC and MSC appears to exist throughout ontogeny: During embryonic development the temporal and spatial distribution pattern for HSC and MSC shows co-localization of MSCs to the major hematopoietic territories as these shift from the aorta-gonad-mesonephron to the liver and finally to the bone marrow, suggesting that mesenchymal progenitors expand within these hematopoietic sites as development proceeds (141, 142).

MSCs can also contribute to the formation of a pathological hematopoietic niche. In particular, MSCs isolated from bone marrow of multiple myeloma patients induced overgrowth of MOLP-6, a stromal cell-dependent myeloma cell line, whereas their capacity to differentiate into the osteoblastic lineage was impaired (143). Understanding the biology of the normal versus abnormal hematopoietic niche will identify novel therapeutic targets. This will be discussed in great details in the review by Kipps *et al.* in this issue.

9. MSCS AND CANCER

Since MSCs are specifically attracted by cancers (90, 144), they seem an ideal vehicle for the selective delivery of chemotherapeutics that are too toxic for systemic administration or that have too short a half-life in the circulation. MSCs engineered to express cytotoxic cytokines have been successfully used in a number of tumor models (144-146). This strategy must be carefully approached, however (147, 148), because the same properties of MSC that make them ideal for tissue repair including their ability to produce angiogenic, trophic and anti-inflammatory factors and their immune-suppressive properties combined with their tropism for tumors, may have dire consequences in the context of cancer. There is amounting evidence that MSC can contribute to cancer progression, metastasis and resistance to chemotherapy by providing a favorable microenvironment.

Cancers are seen by some as wounds that never heal, and it is not surprising in this scenario that MSCs home to tumor sites as they would to any kind of tissue injury. Stromal cells, - primarily cancer associated fibroblasts (CAFs), which are derived from MSCs - –

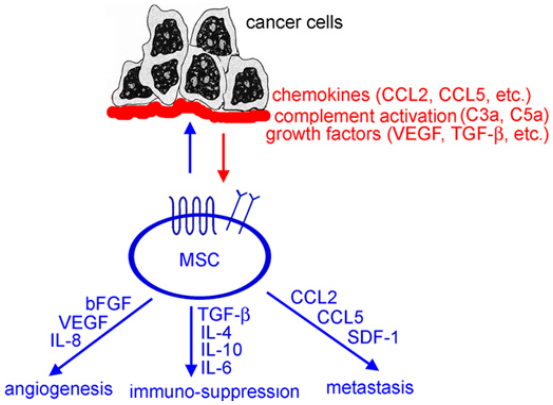

Figure 4. MSCs and the cancer niche: Cancers produce many factors – shown in red – that are chemo-attractants for MSCs, since MSC express a large number of growth factor receptors (e.g. for bFGF, TGF-beta, PDGF, etc.) – shown as a membrane-spanning dimer - as well as G-protein-coupled seven membrane spanning receptors for chemokines, C3a, C5a, etc., also depicted schematically in the plasma membrane of the MSC. In turn, MSCs recruited by these chemoattractants and growth factors produce a variety of factors that support cancer growth, because they are angiogenic, promote metastasis or are immuno-suppressive. A limited selection of these factors is shown in blue.

exploit the normal wound healing process to benefit the epithelial tumor cells, and there is paracrine crosstalk between tumor and stromal cells leading to the deposition of growth factors, cytokines and chemokines produced by the stroma cells that accelerate tumor growth (149-151).

Recently evidence has been accumulating indicating that MSC are recruited to the stroma of developing carcinomas (150, 152, 153). These MSCs may be derived from bone marrow or from resident tissue and once they have engrafted into the stroma of tumors, they contribute to tumor progression and metastasis through the production of growth factors, cytokines and immunomodulatory mechanisms, which has been shown for breast cancer in particular (150, 154-156). MSC engraftment is further increased in cancers exposed to radiation (125). A number of pro-inflammatory peptides (RANTES/CCL5, MCP1/CCL2 and LL-37, which activates the formyl peptide receptor) have been recognized as factors that recruit MSC to breast and ovarian cancers (150, 157, 158). However, this is still an understudied area of research, and understanding of the interplay between tumor cells and MSCs deserves more investigation. MSC recruited to carcinomas differentiate to CAFs (153), which are known to promote tumor growth (149), angiogenesis (159) and metastasis (150). CAFs, the major cellular component of the tumor stroma, have a more profound influence on the development and progression of carcinomas than was previously appreciated (149, 160-163), and a stroma-related gene signature predicts resistance to chemotherapy in breast cancer patients (164). This stromal effect can be direct or due to modulation of the tumor microenvironment, where MSCs create an immune-tolerant milieu (165).

MSCs contribute to cancer cell survival and tumor resistance to chemotherapy by various means (166), which range from non-specific metabolic support (167) to specific protection from asparaginase cytotoxicity in acute lymphoblastic leukemia (ALL) (168).

Nevertheless, there have also been reports that MSCs inhibit tumor growth (169, 170), although the mechanisms involved have not been elucidated. Clearly, more systematic and mechanistic studies are necessary to understand how MSC influence cancer growth and metastasis.

The cancer promoting properties of MSCs do not exclude that MSCs could be used as site-directed delivery tools for cytotoxic agents in cancer, but it has to be pointed out that this is a risky approach, in which the ability of MSCs to contribute to the generation of the cancer cell niche and further disease progression should be considered.

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Abbreviations: BMP-Bone Marrow Morphogenetic Protein, CAF-Carcinoma Associated Fibroblasts, ECM-Extra-Cellular Matrix, EGF-Epidermal Growth Factor, ERK1/2-Extracellular Signal Regulated Kinase, ESC-Embryonic Stem Cells, FGF-Fibroblast Growth Factor, HA-Hyaluronic Acid, HSC-Hematopoietic Stem Cells, iPC-Induced Pluripotent Stem Cells, LIF-Leukemia Inhibitory Factor, MAPK-Mitogen Activated Protein Kinase, MIP-Macrophage Inflammatory Protein, MSC-Mesenchymal Stem Cell, MMP-Matrix degrading Metallo-Protease, PDGF-Platelet Derived Growth Factor, PKC-Protein Kinase C, PODXL- Podocalyxin-like protein, SCF-Stem Cell Factor, SDF-Stromal cell Derived Factor, STAT-Signal Tranducers and Activators of Transcription, TGF-Transforming Growth Factor, VCAM-Vascular Cell Adhesion Molecule, VEGF-Vascular Endothelial Growth Factor

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