Stem Cell Rev (2007) 3:239–248
DOI 10.1007/s12015-007-9001-1

Mesenchymal Stem Cell Preparations—Comparing Apples and Oranges

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Published online: 18 September 2007
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Abstract Mesenchymal stem cells (MSC) represent a type of adult stem cells that can easily be isolated from various tissues and expanded in vitro. Past reports on their pluripotency and possible clinical applications have raised hopes and interest in MSC. Multiple designations have been given to different MSC preparations. So far MSC are poorly defined by a combination of physical, phenotypical and functional properties. As MSC could be derived from different tissues as starting material, by diverse isolation protocols, cultured and expanded in different media and conditions, the MSC preparations from different laboratories are highly heterogeneous. All of these variables might have implications (1) on the selection of cell types and the composition of heterogeneous subpopulations; (2) they can selectively favor expansion of different cell populations with totally different potentials; or (3) they might alter the long term fate of adult stem cells upon in vitro culture. The recent controversy on the multilineage differentiation potentials of some specific MSC preparations might be attributed to this lack of common standards. More precise molecular and cellular markers to define subsets of MSC and to standardize the protocols for expansion of MSC are urgently needed.

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Keywords Mesenchymal stem cell · Culture conditions · Microenvironment · Differentiation · Hematopoietic stem cells · Cell–cell interaction

What's in a Name...?

The term "mesenchymal stem cell" (MSC) is commonly applied to plastic-adherent cell preparations isolated from bone marrow or other tissues that are positive for several antigens such as CD105, CD73 and CD90, that lack expression of hematopoetic antigens and that are able to differentiate at least into osteoblasts, adipocytes and chondroblasts under specific in vitro differentiating conditions [1, 2]. This term has been popularized in the early 1990s by Caplan [3] but the notion of these cells is based in large part on the work of Friedenstein and coworkers in the 1970s. They have described discrete fibroblast colonies in monolayer cultures of bone marrow, spleen and thymus that could be easily maintained in vitro and that demonstrated differentiation characteristics in vitro as well as in vivo upon their re-transplantation [4, 5]. Initially, these authors used murine material and in later studies they demonstrated that equal numbers of fibroblast colony-forming cells (FCFC) could be isolated from adult rabbits, guinea pigs and humans [5]. Caplan and coworkers have used periosteal cells from young chicks that were introduced into cell culture and subsequently transplanted in athymic mice to test their osteo-chondrogenic differentiation potential. These early experiments have already indicated that the differentiation potential is diminished by many population doublings [6]. During the last decade a vast amount of studies demonstrated multilineage differentiation capacity of MSC (Fig. 1). It is commonly accepted that a rare bona fide mesenchymal stem cell population exists but there is

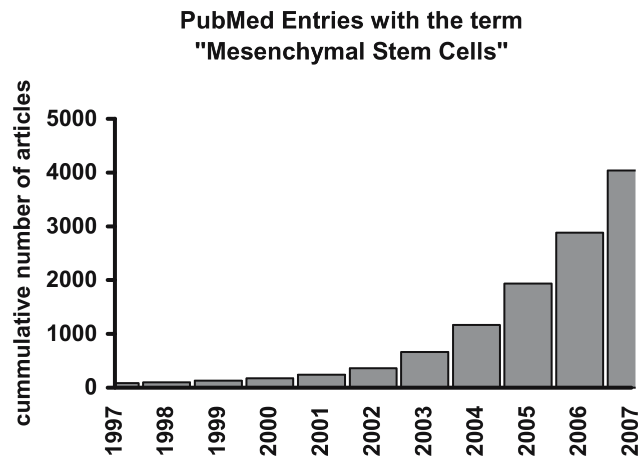

Fig. 1 Emerging interest in "mesenchymal stem cells". The cumulative number of PubMed entries with the search term "mesenchymal stem cells" that have been listed by the end of each year. The number for 2007 has been projected according to the recent development

also evidence that the adherent cell populations are very heterogeneous comprising only a small subset of stem cells. Therefore, it has been suggested to clarify the terminology of these fibroblast-like plastic adherent cells by calling them "mesenchymal stromal cells" [7, 8]. Thereby, the acronym MSC stays the same, whereas the term mesenchymal stem cell should only be reserved for cells that meet specified criteria for stem cells. Alternatively they have been named "multipotent mesenchymal stromal cells" or "multipotent stromal cells" to indicate the multipotent differentiation capacity of these cell preparations [8] The term "mesenchymal progenitor cell" (MPC) has also been used in analogy to the hematopoietic system where hematopoietic stem cells (HSC) are comprised within the CD34-positive cell fraction of hematopoietic progenitor cells (HPC) [9, 10]. A number of groups has described specific protocols for isolation and cultivation of more primitive subsets of MSC with a higher differentiation capacity and the terms "Multipotent Adult Progenitor Cells " (MAPC) [11] or "Unrestricted Somatic Stem Cells" (USSC) [12] have been used. The different nomenclature for MSC would not have been problematic if there were only different terminologies for the same type of cell preparation. We might be using many alternative names for the same type of plastic-adherent cell preparation.

Mesenchymal Stem Cells—Pluripotent, Multipotent or Impotent?

Totipotent stem cells can give rise to all embryonic as well as to extraembryonic cell types and they are produced by the fusion of egg and sperm cell. Pluripotent stem cells are descendants of totipotent cells and can give rise to cell types of all three germ layers. In contrast, multipotent stem cells can only produce a closely related family of cells. MSC resemble a multipotent adult stem cell population which is able to differentiate into different mesodermal cell lineages including osteoblasts, chondroblasts and adipocytes. Albeit controversial, MSC have been reported to differentiate into myocytes and cardiomyocytes and even into cells of non-mesodermal origin including hepatocytes and neurons [11, 13–15]. MAPC have been suggested by the groups of C. Verfaillie to be pluripotent and could provide an alternative to embryonic stem cells (ESC) [11]. From 1998 to 2004, a whole series of reports have been published in highly prestigious journals on transdifferentiation of adult stem cells derived from the marrow. For example hematopoietic cells generated neurons and *vice versa* [16, 17]. It is now believed that in many of these cases adult cell plasticity was in reality misleading signals from fused cells [18, 19].

The group of Catherine Verfaillie described a culture system for MSC that might favor the selection of primitive cells. This MAPC population represents an extremely small proportion of cells that must be kept under strictly defined culture conditions and cell confluence for many passages before they could be established. MAPC have been isolated from mice, rat, swine and human bone marrow [11, 20, 21]. MAPC have been shown to produce cells with characteristics of visceral mesoderm, neuroectoderm or endoderm [13, 22]. Furthermore, MAPC of GFP-transgenic mice could give rise to hematopoietic cells that could be serially transplanted [23]. When injected into an early blastocyst, a single MAPC has been reported to contribute to the development of various tissues [11]. These cells were isolated under defined growth conditions with a low serum content of 2% prescreened fetal bovine serum (FBS was prescreened on the basis of the ability to isolate and maintain high Oct-4 MAPC) supplemented with insulin, transferring, selenium, linoleic acid, ascorbic acid, platelet derived growth factor and epidermal growth factor [13, 21]. Cells were plated in low cell densities on fibronectin coated plastic surfaces. It has been speculated that the described differentiation potential might be attributed to specific selection of a pluripotent stem cell population that might persist in multiple organs after embryonic development. Another explanation is that they represent cells that have been re-programmed and hence de-differentiated [11, 24]. Recently, Kogler et al. have described another subset of MSC derived from human cord blood which they called "unrestricted somatic stem cells" (USSC). These cells were able to differentiate into many cell types including hepatocytes and cardiomyocytes [12]. All these experiments indicated that culture conditions and specific modifications of the isolation protocols might have an enormous impact on the developmental potential of the populations generated, albeit the initial cell population could be

phenotypically identical. On the other hand, the validity of initial experiments has been severely challenged as they could not be reproduced by other groups [24–26]. Reproducibility is crucial for the scientific process and for potential therapeutic applications. It is especially problematic for MSC as slight modifications might lead to completely different cell populations. As long as the questions of their pluripotent differentiation potential are not resolved MSC should be considered as multipotent stem cells. This ongoing controversy and a wide skepticism on the differentiation potential underline the need for a definition of multipotent MSC.

Markers for Prospective Isolation Methods for MSC

Molecular markers and immunophenotype of cell preparations change upon in vitro cultivation and thus it is likely that the phenotype of cultured MSC is very different from primary "colony forming units—fibroblasts (CFU-F)" prior to expansion of MSC preparations. In contrast to MSC, hematopoietic stem cells (HSC) can not be maintained or expanded in vitro without a supportive cellular microenvironment [27]. For HSC research immunophenotypic enrichment thus represents a *conditio sine qua non*. Human HSC can be enriched and characterized by the presence of CD34, CD133, and Thy-1, and they are negative for CD38 as well as many differentiation markers. In contrast, MSC are commonly isolated without specific enrichment. Few markers have been developed for prospective isolation of MSC from primary human and murine tissues. Markers that have been described for positive selection include STRO-1 [28], CD271 (low-affinity nerve growth factor receptor) [29], CD73 (ecto-5′-nucleotidase, SH3, SH4), CD105 (endoglin, SH2) [30], whereas CD45, Ter119 and glycophorin A (CD235) are used for the negative selection of MSC [11, 13]. Recently Buhring et al. described a panel of surface markers including platelet-derived growth factor receptor-D (CD140b), HER-2/erbB2 (CD340), frizzled-9 (CD349) within the CD217-bright population [31]. These markers can be used for enrichment of MSC but the cell fractions are still heterogeneous and the majority of isolated cells will not give rise to CFU-F.

Molecular Markers for Quality Control of Cell Preparations

Preparations of MSC are usually based on in vitro culture and expansion of specific cell populations. Gene expression and surface markers may differ between the original cell population and the expanded MSC product. Therefore characterization of the expanded MSC populations is of utmost significance. Thus far surface markers have failed to serve this purpose. To circumvent this problem the International Society for Cellular Therapy proposed three minimal criteria to define MSC [1]: (1) MSC must be plastic-adherent if maintained in standard culture conditions, (2) MSC must express CD105, CD73 and CD90, and lack expression of hematopoietic markers such as CD45, CD34, CD14 or CD11b, and (3) MSC must be capable of differentiation to osteoblasts, adipocytes and chondroblasts under in vitro differentiating conditions. We have used a panel of 22 surface markers including those mentioned above to analyze human MSC preparations derived from bone marrow, adipose tissue and umbilical cord blood. The immunophenotypic analysis was in line with the literature. However, human fibroblast cell lines (HS68 and NHDF) displayed an identical phenotype and thus could not be discriminated from MSC. These surface markers are not sufficient to define MSC [2]. Osteogenic, adipogenic and chondrogenic differentiation was observed in all MSC preparations, whereas human fibroblasts do not possess this in vitro differentiation capacity [2, 32]. However, in vitro differentiation assays can hardly be standardized as we are dealing with a potentially heterogeneous composition of subpopulations. Hence, these minimal criteria are helpful but further characteristics must be defined to identify a homogeneous population of MSC.

Gene expression analysis has provided another dimension for molecular characterization of cell preparations. We have compared gene expression profiles of MSC from bone marrow, adipose tissue and cord blood in comparison to those of non-multipotent fibroblasts [2]. Initial analysis has demonstrated a consistent up-regulation of 25 well characterized genes in all MSC preparations irrespective of origin or culture conditions. These genes included fibronectin 1 (FN1) and other extracellular matrix proteins (GPC4, LTBP1, ECM2, CSPG2) as well as transcription factors (nuclear factor I/B [NFIB], homeo box genes HOXA5 and HOXB6, inhibitor of differentiation/DNA binding ID1). However, none of these genes alone seems to be specific for MSC and thus, this overlap did not identify a unique MSC marker. Furthermore, we have analyzed the proteome of MSC. One hundred thirty-six protein-spots were unambiguously identified by MALDI-TOF-MS. Most of the identified proteins up-regulated in MSC play a role in cytoskeleton, protein folding and metabolism [33]. Candidate genes should be highly expressed and localized on the cell surface. In contrast, transcription factors and regulators of signal transduction are often scarcely expressed and the use of extracellular proteins is unfavorable for quality control purposes.

Our results indicated that a single genomic or proteomic marker will not be adequate, but rather a combination of markers might be necessary to specify multipotent MSC.

Nevertheless, our data have provided the basis for identifying a panel of up-regulated genes that would serve as a quality control for MSC at a genomic or proteomic level.

A MSC is a MSC is a MSC?

Another constrain is the lack of standardization for preparation of MSC (Fig. 2). The above mentioned inconsistency in the reproduction of some of the initial experiments might be a result of different culture methods. These methods have implications (1) on the selection of cell types as well as the composition of sub-populations; (2) they can selectively favor expansion of subpopulations; and (3) the culture conditions might continuously induce genetic instability.

MSC preparations contain several morphological distinct cell types: spindle-shaped cells, large flat cells and small subpopulations [34]. Heterogeneity of starting populations renders comparison of results between different groups difficult. The lack of molecular standards underlines the importance of standardized isolation protocols and the following factors have to be taken into account:

Species

MSC have been isolated from many different species such as mouse, guinea pig, chick, rabbit, dog, pigs and human. Knowledge gained from animal models cannot always be extrapolated for human stem cells. There appear to be many similarities with human MSC but a systematic comparison of MSC from different species is jet elusive. Human MSC age after approximately 40 population doublings, whereas senescence of rat MSC has not been reported [35]. Experimental data of MSC from animal models has to be validated in the human system prior to clinical application. For the rest of this review we will focus on human MSC.

Isolation from Different Tissue

MSC were originally isolated from bone marrow [32, 36], but similar populations have been reported in other tissues such as adipose tissue [37], umbilical cord blood [38], peripheral blood [39], connective tissues of the dermis and skeletal muscle [20]. Furthermore, cell preparations that fulfill the minimal criteria for MSC have also been isolated from other tissue of adult mice such as brain, liver, kidney, lung, thymus and pancreas [40]. There is no doubt that multipotent cell populations of mesenchymal derivation will reside in many tissues. Our gene expression analysis has provided clear evidence that a significant number of genes is differentially expressed in MSC isolated from specific tissue [2]. Correspondingly, the differentiation potentials and functional implications varied widely among MSC preparations derived from different origins [27, 41].

Isolation/Depletion using Surface Markers

Various surface markers such as STRO-1, CD271, CD73, CD105 have been used for positive selection of MSC. Alternatively, negative selection was performed using hematopoietic surface markers such as CD45, Ter119 and glycophorin. These markers have been used alone or in combination. They can be used to enrich for fibroblast colony forming units (CFU-F) but they do not enable direct isolation of multipotent MSC. A sophisticated comparison of the molecular features of MSC that were isolated with different enrichment methods is elusive, but it is likely that the composition of heterogenic cell preparations is affected by these separation steps.

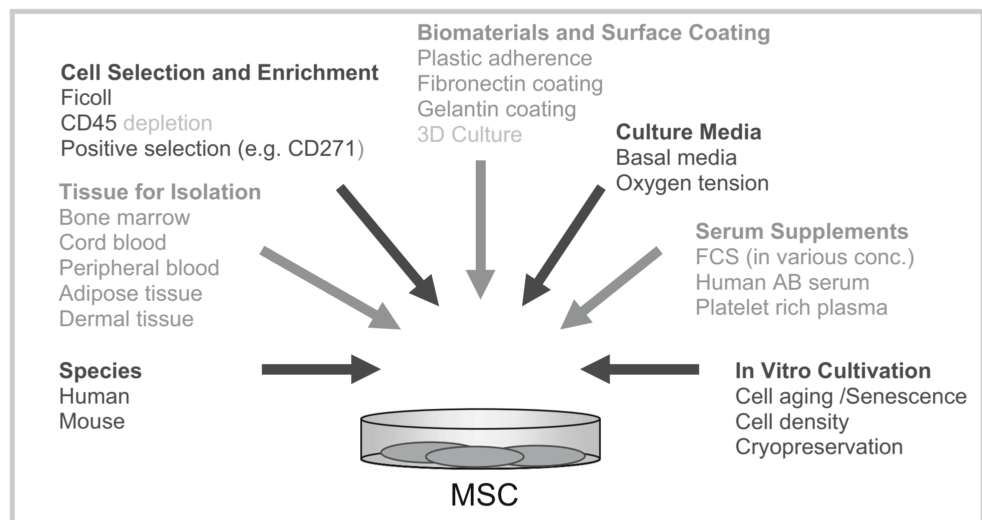

Fig. 2 Critical parameters for MSC isolation. Various different culture isolation protocols for MSC preparations have been described in different studies. Each of these parameters has impact on the composition of cell preparations and needs to be taken into account

Protein Coating of Surface and Biomaterials

Adherence to the surface of culture dishes is the most prominent feature of MSC. Properties of the surface (e.g. roughness, hydrophobicity and elasticity) significantly affect selection or differentiation potential of cell preparations [42, 43]. Many protocols have used additional protein coating (e.g. fibronectin, gelatine or collagen) to enhance cell adhesion and to mimic certain aspects of the natural extracellular microenvironment. Culture on either fibronectin or gelantine greatly affects the morphology of the cell products after culture. Furthermore, three-dimensional scaffolds of extracellular matrix derived biomaterials have been used as carriers in transplantation models or as infiltration matrices for MSC. For examples collagen and fibrin are clinically well-established and approved matrices for wound healing. Such 3D-scaffolds of bio-materials have also been used for expansion of MSC.

Culture Conditions

We have previously demonstrated that culture media have a tremendous impact on gene expression and proteome of MSC [2, 33]. A huge arsenal of basal culture media is available and many different media have successfully been used for isolation of MSC in different laboratories. Furthermore, there is evidence that oxygen tension plays an important role and that hypoxia accelerates MSC differentiation [44].

Serum Supplements

So far there are no reliable culture isolation protocols for MSC preparation without serum additives. Serum concentrations usually vary between 2 and 20%. Most studies have used fetal calf serum (FCS). Concerns regarding BSE, other infectious complications and host immune reactions have fueled investigation of alternative culture supplements. Recently processing techniques have been described that are based on alternative reagents of human origin (serum, plasma, platelet rich plasma) [45–48]. The impact of these supplements on the composition of cell preparations is yet unknown but different growth kinetics and cell morphology indicate their relevance (Fig. 3a). Therefore, the development of a chemically defined and serum free growth medium is essential for standardized MSC preparations.

In vitro Cultivation (Passage, Density and Cryopreservation)

MSC can be passaged *in vitro* for a limited number of times (about 8–15 passages equivalent to 25–40 population doublings) before they become senescent and stop proliferation. The cells proliferate slower, become larger and less tightly packed (Fig. 3b). The molecular mechanisms of this “Hayflick limit” are yet unknown. Certainly, molecular profiles and functional features of MSC are affected by this process of cellular aging [49–51]. Cell density of cultures seems to be crucial, too. Once grown to confluency, MSC have been shown to lose some of their differentiation

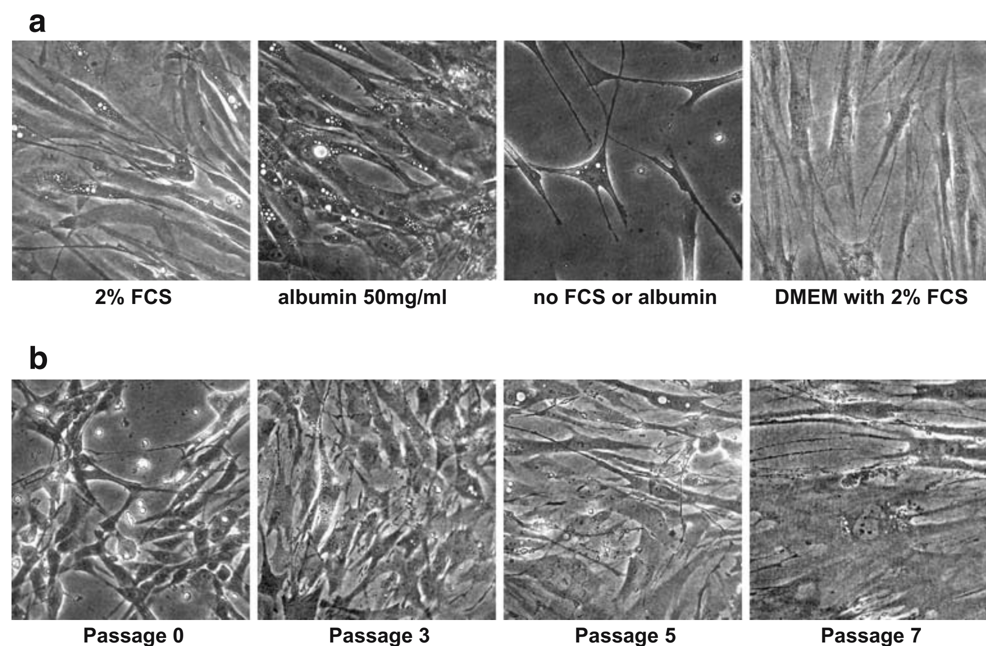

Fig. 3 Impact of culture conditions on cell morphology. MSC were isolated from bone marrow as described before [2, 13]. Cells of the fifth passage were simultaneously cultivated under (1) standard growth media with 2% FCS, (2) standard growth media with 50 mg/ml albumin, (3) standard growth media without FCS, and (4) in DMEM with 2% FCS. Cell morphology is demonstrated after 7 days cultivation (including one additional passage) under different growth conditions (**a**). *In vitro* cultivation over several passages has also impact on cell morphology. 0, 3rd, 5th, and 7th passage of the same MSC preparation from bone marrow is demonstrated (**b**)

potential [13, 34, 52, 53]. Furthermore, MSC are often cryopreserved with DMSO in liquid nitrogen. There is evidence that cryopreserved and non-cryopreserved MSC possess the same differentiation potential but an effect on their biological properties can not be excluded [54, 55].

Donor Variability

MSC are isolated as primary cell preparations from individuals with different genetic background and diseases. Donor variability can hardly be standardized for MSC preparations but it needs to be taken into account.

This enumeration of relevant factors for the composition of MSC preparations indicates that we are currently comparing apples and oranges. The multiple differences in cell preparations can not even be reflected by nomenclature. Instead, precise molecular markers are essential. Specific cell–cell contacts might be a correlate for multipotent MSC.

A Novel Type of Cell-Junctions between MSC

Recently, our group has demonstrated that MSC under in vitro conditions are interconnected by special tentacle-like cytoplasmatic protrusions and invaginations, termed *processus adhaerentes* [56]. Cell junctions connect MSC in the intercellular space with small *puncta adhaerentia*. Cell processes could be traced that made junctional contacts with up to 8 other MSC, and over distances exceeding 400 μm. Alternatively, they can also form deep plasma membrane invaginations in neighboring cells (*recessus adhaerentes*). This novel type of cell junctions is characterized by a molecular complement comprising N-cadherin and cadherin-11, in combination with the cytoplasmic plaque proteins α- and β-catenin, together with p120ctn and plakoglobin, as well as afadin [56]. The long *processus adhaerentes* interconnect several distant MSC to formations of a closer-packed cell assembly. The frequency and morphology of these conjunction complexes are greatly affected by culture conditions (unpublished observation). A similar type of homotypic cell–cell interaction has previously been described by W. Franke and co-workers in studies of primary mesenchymal cells of the mouse embryo [57]. These findings implicate that this novel type of cell junctions is more wide spread in embryonal and other tissues and they might be relevant for the primitive function of MSC and heterotypic interaction with other cell types.

MSC as a Model System for the “Stem Cell Niche”

Heterotypic interaction of stem cells with cellular determinants of the stem cell “niche” is crucial for the regulation of self-renewal and differentiation [58, 59]. We and other groups have demonstrated the supportive nature of MSC to provide a cellular microenvironment for hematopoietic stem cells (HSC) [60, 61]. In the murine model specialized spindle-shaped N-cadherin-expressing osteoblasts (SNO) located in the endosteum were postulated to be essential components of the HSC niche [62]. Furthermore, other cell types such as osteoclasts, stromal and endothelial cells as well as extracellular matrix represent components of the niche [63, 64]. This supportive interaction is mimicked by in vitro model systems by co-culture of HPC with supportive feeder layer cells. Many stroma cell preparations, including MSC, have been shown to maintain HPC in an undifferentiated state with varying degrees of efficiency [65–69]. Although numerous studies have demonstrated the vital role of stroma feeder layers for maintenance of multi-potency of HPC in vitro [67, 70–72], little is known about the precise cellular and molecular mechanisms of this interaction. We have demonstrated that HPC actively migrate towards stromal cells and adhere to the latter [73]. Adhesion of HPC, maintenance of a primitive immunophenotype and maintenance of LTC-IC was always higher on MSC isolated from bone marrow and cord blood compared to MSC from adipose tissue [27]. These findings indicate that there are functional differences between MSC preparations from different tissue in their hematopoietic supportive function. We have also demonstrated that MSC from bone marrow and cord blood are superior to fibroblasts for maintenance of primitive function of HPC [27]. Thus, MSC provide an artificial model system for the stem cell niche to investigate the molecular mechanisms that regulate asymmetric cell division in HSC [59].

Cell Connections Govern Cell Fate

The essential role of direct cell–cell contact for the regulation of self-renewal and differentiation of adult stem cells has been shown in various cell systems. Specific junctional complexes play a similar role in the hematopoietic system [74]. We have demonstrated that blocking antibodies for ITGA5, ITGB1 and CD44 reduced adhesion of HPC to MSC feeder layer and that ITGB1 is involved in the maintenance of self-renewal upon interaction [60, 61, 75]. Furthermore, we have analyzed gene expression profiles of MSC with regard to the functional differences in their hematopoiesis supportive capacity [27]. Genes up-regulated in MSC preparations with the ability to maintain stemness included cadherin-11, N-cadherin, integrins alpha-1 (ITGA1), alpha-5 (ITGA5, CD49e) and beta-1 (ITGB1, CD29), VCAM1, NCAM1 and thrombospondin 1 (THBS1) [61].

To examine the sequel of cell–cell contact on the HSC we have previously examined adherent and non-adherent

fractions of $CD34^+$ cells upon interaction with MSC from bone marrow [60]. Gene expression analysis revealed that cadherin-11, VCAM1, thrombospondin 2, ITGBL1, and CTGF were among the genes with highest over-expression in the adherent fraction of $CD34^+$ cells. It is intriguing that the same adhesion molecules are highly expressed in adhesive feeder layers as well as in the adherent fraction of HPC. These results imply that molecular mechanisms essential in maintenance of "stemness" are mediated by an orchestra of cell–cell junction proteins (cadherin-11, N-cadherin, NCAM1, VCAM1) and cell–matrix junction proteins (ITGA5, ITGB1).

Secretory Function of MSC

MSC secrete a variety of cytokines and growth factors that have both paracrine and autocrine activities [76]. Indeed, all cells secrete various bioactive agents that reflect both their functional status and the influence of their microenvironments. For MSC, analysis of secretory profiles is of specific relevance as secreted molecules might affect direct and indirect effects: direct effects on the MSC preparation themselves, indirectly by inducing other cells in the vicinity to alter their biological properties and functions. Such indirect or trophic effects of MSC might explain some of the positive therapeutic effects observed with MSC without any evidence for "transdifferentiation" of MSC. For example, such trophic effects have been proposed in treatment of stroke, myocardial infarct and meniscus repair [76].

We have studied the cytokine production of MSC by cytokine antibody arrays, ELISA and by a cytometric bead array [27]. There were reproducible differences in the chemokine secretion profiles of various MSC preparations but there was no clear concordance with differences in their potential to maintain primitive function of HPC. The lack of consistency of different hematopoietic supportive function of MSC with their chemokine secretory profile underlines the significance of direct cell–cell contact between HPC with very specific cellular determinants in maintaining "stemness", and that human MSC are not just more efficient fibroblasts.

The Potency of MSC in Clinical Application

Theoretically, MSC could be isolated from a small aspirate of bone marrow or tissue samples and readily expanded in vitro and thus, they might be of potential for regenerative medicine. A look at the website: http://www.ClinicalTrials.gov of the United States National Institute of Health provides information on the current clinical trials based on the use of MSC. The spectrum of clinical applications includes treatment of steroid refractory graft versus host disease (GVHD), peridontitis, severe chronic myocardial ischemia, distal tibia fractures, decompensated liver cirrhosis, multiple sclerosis, tumor induced osteomalacia and Crohn's disease. Thus far there were hardly any reports on the side effects of clinical application of MSC and some of the preliminary observations appeared promising. The beneficial effects of MSC administration were in some studies probably not associated with cell replacement and differentiation through MSC. For example MSC based myocardial therapy has proceeded at a rapid pace and there is sound evidence for successful cardiac regeneration or repair upon MSC treatment [77]. This effect might be attributed to (1) differentiation of the administered cells into all of the cellular constituents of the heart, (2) release of factors capable of paracrine signaling, (3) fusion of the administered cells with the existing constituents of the heart, or (4) stimulation of endogenous repair by injected cells [78, 79]. So far only differentiation towards mesodermal cell types such as osteocytes, chondrocytes and adipocytes could be proven in vitro and in animal models.

The lack of knowledge on the precise mechanisms might not prevent their applications in the clinical setting if there are benefits for the patient and if there are no or minimal side effects.

Conclusion

Recently the International Stem Cell Initiative has characterized 59 human embryonic stem cell lines from 17 laboratories worldwide [80]. The goal of this study was to assess the similarities and differences in the expression of commonly used markers for hESC and to identify a set of well-validated markers to establish ESC identity. All cell lines exhibited similar expression patterns for several markers of human ESC but they were not identical. A similar comparative approach would be equally important for adult stem cells especially for MSC. In contrast to ESC, human MSC have already found their way into the clinic and might represent a potential chance of cure for some degenerative disorders. The lack of common criteria and universal standards for preparation of MSC has greatly hampered further progress. Furthermore, functional characterization of MSC is limited by the available methods for in vitro differentiation. There is an urgent need for a comprehensive view of the mesenchymal stem cell identity and characteristics.

Acknowledgements The authors thank Patrick Horn and Anke Diehlmann for their support in MSC culture and photo documentation. This work was supported by the German Ministry of Education and Research (BMBF) within the National Genome Research Network NGFN-2 (EP-S19T01) and within the supporting program "cell based

regenerative medicine" (START-MSC), the German Research Foundation DFG (HO 914/7-1), the Joachim Siebeneicher-Stiftung and the Academy of Sciences and Humanities, Heidelberg (WIN-Kolleg).

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