

Characteristics of Mesenchymal Stem Cells – New Stars in Regenerative Medicine or Unrecognized Old Fellows in Autologous Regeneration?

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Key Words

Mesenchymal stem cells · Marrow stromal cells · Characterization · Differentiation · Regeneration

Summary

For years mesenchymal stem cells (MSC) have been in the focus of research in the emerging field of regenerative medicine. Due to the heterogeneity of cells with MSC-like properties their comprehensive characterization is necessary. In the following, issues of nomenclature, basic characterization, sources, stemness, and therapeutic potential of MSC are discussed, highlighting some aspects in the rapidly expanding field of MSC research.

Introduction

When Alexander Friedenstein and colleagues [1, 2], in the 1970s, described fibroblastoid cells which were obtained from bone marrow (BM) showing colony formation and in vitro as well in vivo osteogenic differentiation potential, it hardly could be predicted that in the following decades the emerging field of stem cell research would focus on cells with similar properties.

Although extensive investigations, from basic stem cell research up to clinical trials, on mesenchymal stem cells / marrow stromal cells (MSC) have been performed by various groups, the knowledge about these cells remains incomplete. The complexity of research on MSC is, among other reasons, based on the fact that numerous subpopulations with more or less MSC-like properties are grouped under the roof of

Schlüsselwörter

Mesenchymale Stammzellen · Knochenmarkstromazellen · Charakterisierung · Differenzierung · Regeneration

Zusammenfassung

Seit einigen Jahren befinden sich die mesenchymalen Stammzellen (MSC) im Fokus der Forschung im Bereich der regenerativen Medizin. Aufgrund der Heterogenität der Zellpopulationen, die MSC-artige Eigenschaften aufweisen, ist deren umfangreiche Charakterisierung notwendig. Der folgende Artikel befasst sich mit der Nomenklatur, der grundlegenden Charakterisierung, den Quellen, dem Stammzellcharakter sowie dem therapeutischen Potential der MSC. Hierbei sollen einzelne Aspekte im Bereich der sich rasant entwickelnden MSC-Forschung schlaglichtartig beleuchtet werden.

‘MSC’. Therefore, carefully characterizing the cells as MSC which is the initial task before continuing research is time consuming but obligatory.

In the following, the characteristics of MSC which are accepted as essential to date and the possible therapeutic potential of MSC will be discussed. This implies issues of differentiation, paracrine activity and immunomodulation.

Basic Characteristics of Mesenchymal Stem Cells

Recently some interesting molecules were described which may be useful to identify MSC (sub)populations (CD271, GD2, CD49a, W7C5, W8B2, C15, CDCP1, CD340, CD349, SSEA1/4) [3–7]. However, a distinct exclusively MSC-restricted marker has yet to be identified. Furthermore, the cellular

The potential of MSC

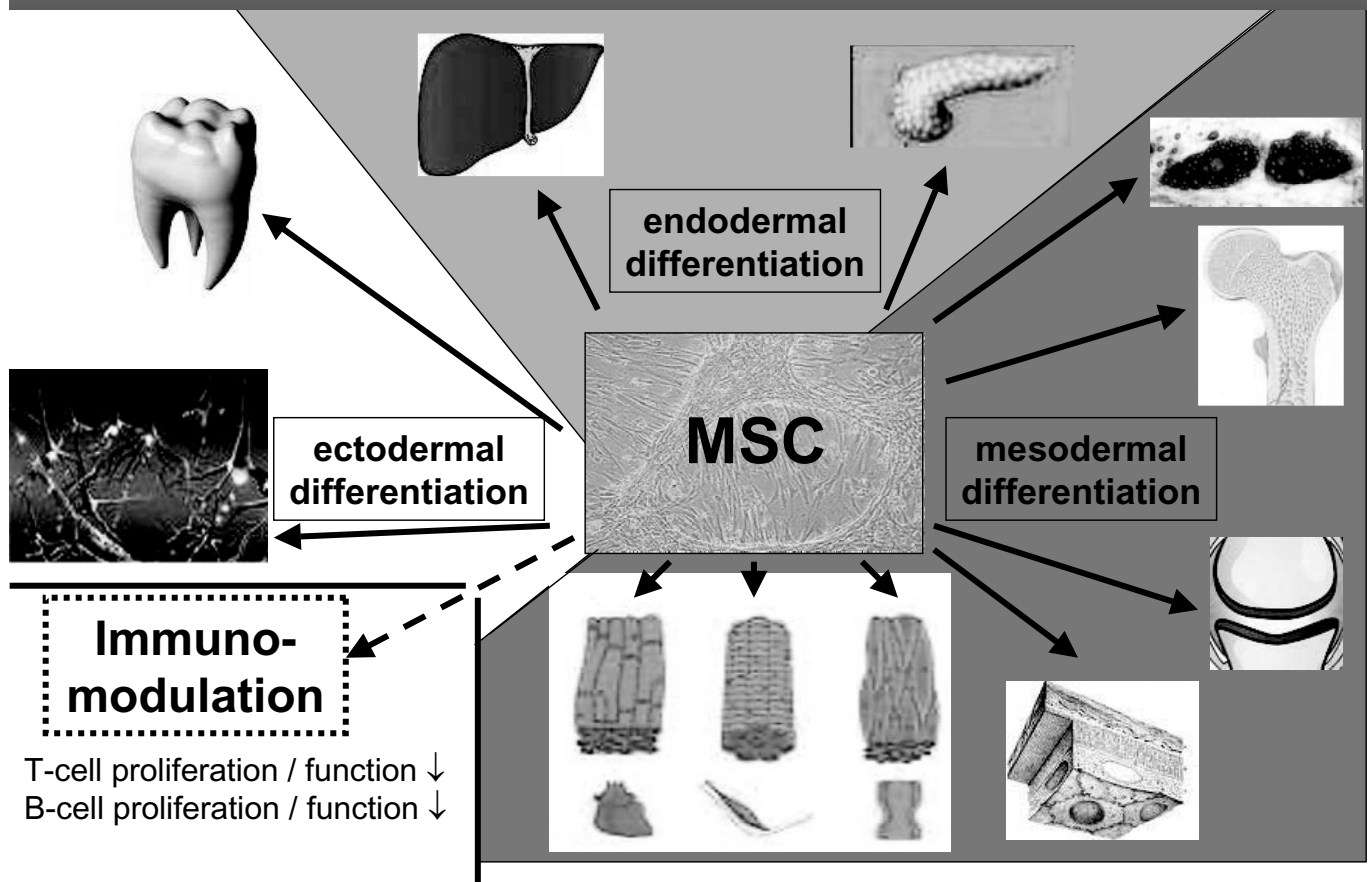


Fig. 1. The differentiation potential (differentiation in cells of mesodermal, endodermal and ectodermal lineage) and immunomodulatory potential of MSC.

shape by itself is not sufficient to determine the cells because the morphology of MSC may vary from spindle shape to broad trapezoid shape depending on culture conditions and passages [8]. Due to the heterogeneity of MSC-like cell populations and biased sample preparations [9, 10], it is necessary to standardize the characterization of MSC. For this purpose, a useful approach is application of the minimal criteria for defining MSC which have been published by the International Society for Cellular Therapy (ISCT) [11]. These defining criteria are based on phenotypical and functional issues of cultured MSC:

Surface Antigen Pattern

Concerning the characterization of the cellular surface, it has to be emphasized that the following antigen pattern is characteristic for human MSC only. MSC isolated from other species, particularly mouse, may show a different pattern which varies among the strains [12]. Moreover, the surface antigen pattern fails to reflect the developmental potential of the MSC [9].

The typical surface antigen pattern of cultured, nonstimulated and/or not differentiated MSC (positive antigen expression is defined $\geq 95\%$ positive counts, negative antigen expression is defined $\leq 2\%$ positive counts by flow cytometry) comprises:

- positive antigens: CD73, CD90, CD105,
- negative antigens: CD14 or CD11b, CD34, CD45, CD79 α or CD19, HLA-DR.

Multipotent Differentiation Potential

Multipotent differentiation is defined as the ability to differentiate into different types of cells/tissue but not to all tissues of the body nor many of the cells that support the pregnancy (pluripotent) or even give rise to a new individual (totipotent) [13]. Cells defined as MSC should show the ability to differentiate into adipogenic, osteogenic and chondrogenic lineage (tri-lineage differentiation potential) after treatment with the respective differentiation media [11].

The in vitro differentiation is usually determined by specific

staining techniques: MSC in adipogenic differentiation show lipid vacuoles which can be stained with oil red O. Osteogenesis is shown e.g. by staining for alkaline phosphatase and calcium. Glycosaminoglycans in pellets of MSC undergoing chondrogenic differentiation can be stained with toluidine blue [14].

The ISCT definition of MSC requires the ability of plastic adherence. Accounting for reports on nonadherent MSC subpopulations [15, 16], plastic adherence may not be an essential aspect of MSC characterization.

It should be pointed out that these issues of characterization are the result of pure *in vitro* investigations. This implicates that the characterization and comparability of cell populations in respect to their MSC-like properties *in vitro* is feasible. However, there is evidence that the transferability of the *in vitro* characteristics into the *in vivo* situation is limited. The expression of CD45 and CD29 on MSC, for example, is completely different depending on the environment which surrounds the MSC: Freshly isolated MSC express high amounts of CD45 but do not express CD29 on their surface, under culture conditions CD45 is quickly lost, whereas CD29 is up-regulated [17]. Moreover, the antigen expression can be influenced e.g. by stimulation with IFN- γ (up-regulation of HLA-DR) [11] or malignant transformation (loss of CD90) [18]. Antigens that are often detected on human MSC such as CD166 can also be detected on other cell types like malignant melanoma cells [19].

The Nomenclature of Mesenchymal Stem Cells

Fortunately, the acronym MSC stands for ‘mesenchymal stem cells’ as well as for ‘marrow stromal cells’. At least relating to the BM as source, both terms describe a similar cell population depending on the criteria of definition: Adult ‘mesenchymal stem cells’ are defined as multipotent cells which can differentiate into mesenchymal and nonmesenchymal lineages. ‘Marrow stromal cells’, showing a similar differentiation potential, are located in the BM where they interact with hematopoietic stem cells (HSC) supporting them in their niche [14, 20–22]. Other acronyms defining nonhematopoietic stem/progenitor cells with MSC-like properties stand for ‘multipotent adult progenitor cells’ (MAPC) and ‘marrow isolated adult multilineage inducible’ (MIAMI) cells [23]. For clarity’s sake and because MSC cannot be isolated exclusively from the BM, in the following the acronym ‘MSC’ is used in general defining ‘mesenchymal stem cells’.

Sources of Mesenchymal Stem Cells

MSC have been isolated from various fetal and postnatal organs and tissues, including brain, spleen, liver, kidney, lung, muscle, thymus, pancreas, adipose tissue, blood vessels (aorta

artery, vena cava, small vessels from kidney glomeruli) and umbilical cord blood [24, 25], from various species (humans, rats, mice, cats, dogs, rabbits, pigs, baboons) [8]. Their distribution throughout the organism seems to be related to their prevalence in a perivascular niche [24]. Up to now, it is common assumption that they are usually (i.e. in the absence of pathological status or mobilization procedures [26]) not or only in very low numbers present in adult human peripheral blood [24], but there are reports on isolation of MSC from the peripheral blood of rats [27] and pigs [28]. However, the most common source of MSC is BM. From this compartment, MSC can be isolated in comparatively high numbers and with sufficient differentiation potential. Although a number of different methods has been described for isolating MSC (including antibody- or aptamer-based negative or positive enrichment/depletion techniques and culture-based selection methods [10, 29]) the most frequently applied technique is the selection of MSC by plastic adherence using the affinity of MSC to plastic surfaces [14]. However, with this technique a contamination with other adherent cells without MSC-like properties is unavoidable. The frequency of MSC in the bone marrow is up to 0.01% of the mononuclear cells after plastic adherence [3, 30, 31]. It has to be pointed out that MSC preparations from different species (human, mouse, rat) and sources (BM, adipose tissue or umbilical cord blood) may vary in their surface epitope pattern, differentiation or proliferation capacity [12, 25, 32].

Mesenchymal ‘Stem’ Cells?

Referring to this question, Javazon et al. [8] pointed out that, due to their proliferative potential, their clonal regeneration and their differentiation potential, MSC may fulfill the current criteria of stem cells (capacity for self-renewal and ability to give rise to one or more types of differentiated progeny). In contrast to embryonic stem cells or tumor cell lines, adult MSC or HSC show senescence *in vitro* due to the declining activity of telomerase [8, 13, 33]. The subsequent telomere shortening is regarded as a mechanism in long-lived mammals, avoiding unlimited and life-threatening proliferation of organ-specific stem cells [13]. It is reasonable to ascribe MSC a kind of ‘stemness’ in the first place because of their multipotentiality and capacity of transdifferentiation [34]. According to the morphological and phenotypical heterogeneity of MSC, there is a functional heterogeneity among MSC. MSC populations of different donors for example vary in their growth properties and osteogenic differentiation potential [35, 36]. Moreover, the tri-lineage differentiation potential could only be ascribed to a minority of clonal MSC preparations [37], and culturing of MSC leads to a loss of multi-potentiality [38].

Beyond the tri-lineage mesodermal differentiation, MSC can *in vitro* and *in vivo* transdifferentiate into cells which show phenotypical and functional properties of the neuroectodermal lineage like astrocytes or neurons [39, 40]. However these

transdifferentiated MSC cannot be regarded e.g. as neurons because they lack of the neuron-typical properties such as formation of functional filaments or adequate electrophysiological behavior [7]. More in vitro and in vivo experiments demonstrate the broad plasticity of MSC: There are numerous reports on differentiation of MSC into cells with myogenic or cardiomyogenic properties in vitro [41, 42]. After transplantation, MSC had been shown to differentiate in vivo into various epithelial cell types, e.g. pneumocytes, myofibroblasts, retinal pigment epithelial cells, skin epithelial cells, sebaceous duct cells as well as renal tubular epithelial cells [7].

Regeneration by Mesenchymal Stem Cells

In disease models like myocardial infarction, cerebral ischemic stroke, pulmonary fibrosis, nephropathy and osteogenesis imperfecta systematically administered MSC engraft preferentially to the site of injury [14]. These observations and their differentiation potential soon led to the mechanistic assumption that MSC may represent ideal candidates for tissue regeneration. Actually, the application of MSC resulted in significant clinical improvement in various diseases (osteogenesis imperfecta, lung injury, kidney disease, diabetes, myocardial infarction) and neurological disorders (cerebral ischemia) and other diseases of the central nervous system, including neurodegenerative and inflammatory disorders [7, 43–45]. Interestingly, the therapeutic effects of MSC seem not necessarily require the in vivo differentiation of the MSC. Moreover, only a minority of the MSC shows long-term survival after transplantation [46]. Therefore, it is reasonable to assume that a paracrine activity of MSC (e.g. the secretion of growth factors and other cytokines) leads to tissue regeneration [7, 14, 47, 48]. Due to the gap between the low survival and in vivo transdifferentiation rates and the evident clinical effects, the paracrine activity of MSC could possibly be regarded as the more relevant mechanism for tissue repair as compared to transdifferentiation [7]. Referring to autologous regeneration, in all probability MSC act as an internal cellular resource in order to repair damaged tissue. This hypothesis is supported by the following observations: i) MSC of fetal origin can be detected in maternal tissues long time after pregnancy, participating in tissue regeneration [8]; ii) BM-residing MSC can be mobilized after tissue injury – these cells had been shown to participate in the regeneration of myocardial infarction, skeletal muscle damage or cerebral stroke [23].

Immunomodulation by Mesenchymal Stem Cells

In vivo observations after administration of MSC like improvement of the outcome after allogeneic transplantations by promotion of hematopoietic engraftment and amelioration of graft-versus-host disease [14, 49] as well as the amelioration of inflam-

matory response and improved clinical course in experimental allergic encephalomyelitis [50] are impressive proofs for the immunomodulatory properties of MSC. Usually, immunomodulation by MSC comes as immunosuppression. This is the result of interactions of MSC with T and B cells: T-cell proliferation and function is suppressed, B-cell proliferation, differentiation and chemotaxis is inhibited [14]. Moreover, MSC have been shown to suppress allospecific antibody production in vitro [51]. At the molecular level, immunomodulation by MSC is mediated e.g. by indoleamine 2,3-dioxygenase (IDO) [52]. IDO, induced by IFN- γ , catalyzes the metabolism of tryptophan. The tryptophan depletion results in inhibiting the growth and function of T cells [53]. Alternatively, immunomodulation by MSC can be mediated by insulin-like growth factor(IGF)-binding proteins [54], prostaglandin E2 [55] or HLA-G [56].

Malignant Transformation of Mesenchymal Stem Cells and Interactions with Tumor Cells – the Other Side of the Coin

Despite the options of MSC-based therapy for the treatment of diseases and regeneration of the aged organism, some safety aspects have to be addressed:

There are emerging reports, although controversially discussed [33], on malignant transformation of cultured MSC [18, 57]. Therefore, appropriate tools for the surveillance of MSC cultures have to be developed. Due to the immunosuppressive properties of MSC the question came up whether these cells may promote tumor growth in vivo. Actually, there is evidence that MSC, after systemic administration, home to neoplastic tissue [58–60]. Moreover, MSC are involved in tumor growth in vivo and promote metastasis [61–63].

Finally, trying to answer the question whether MSC are the new stars in regenerative medicine or unrecognized old fellows in autologous regeneration, we may return to history again: Julius Friedrich Cohnheim speculated in the 19th century about the role of BM-derived fibroblasts in wound healing [64]. In the following, numerous investigators worked on the possible transformation of peripheral blood-derived monocytes to macrophages and fibroblasts [65]. Recently, Pufe et al. [66] reported on ‘new’ adult pluripotent cells derived from human peripheral blood monocytes identified as programmable cells of monocytic origin (PCMO). These cells show multilineage potential comparable to that of MSC. To date it is unclear whether these multipotent, monocyte-derived cells belong to the MSC family or not. Moreover, BM-derived MSC, synovial fibroblasts, dermal fibroblasts and lung-derived fibroblasts which are summarized as ‘stromal cells’ share not only a common cell surface marker phenotype, but they can also be differentiated in vitro and they are able to inhibit the proliferation of peripheral blood mononuclear cells following polyclonal stimuli indicating a comparable immunosuppressive potential [67]. These results indicate that rather extensive-

ly characterized and assumed to be well-known cells exhibit properties which are up to now accredited to nonhematopoietic stem or progenitor cells. Possibly it depends on the point of view whether MSC represent a distinct entity of progenitors or whether they are multipotent subpopulations of long 'known' cell types like tissue macrophages or fibroblasts.

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