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Biology and applications of mesenchymal stem cells

*PEDRO CESAR CHAGASTELLES, NANCE BEYER NARDI AND MELISSA CAMASSOLA**

ABSTRACT

Undifferentiated adult stem cells are responsible for cell replacement in adult organisms. Initially isolated from the bone marrow, they are now known to be distributed throughout the organism as a whole, with a perivascular location. They are defined by properties which include proliferation as adherent cells, a defined immunophenotype, and the capacity to differentiate in vitro *into osteoblasts, adipocytes and chondroblasts. Mesenchymal stem cells (MSCs) are considered as one of the most promising cell types for therapeutic applications. Mechanisms responsible for this therapeutic role are not well understood, and may involve differentiation or, as most evidences point out, paracrine activity. The ability to modulate the immune system opens a wide range of applications, mainly for autoimmune diseases and graft-versus-host disease. Preclinical and clinical studies show promising results, but controversial results are still reported, indicating the need for further basic and preclinical investigation on their therapeutic potential. This review will focus on recent advances in understanding MSC biology and applications in cell therapy.*

Keywords: *mesenchymal stem cells, adult stem cells, cell therapy, biology, applications*

Introduction

By definition, stem cells are able to replicate giving rise to other stem cells and to differentiate into at least one specialized cell type. Stem cells are currently classified according to their origin as embryonic and adult stem cells. Embryonic stem cells (ESCs) are isolated from the inner mass of blastocysts, can be expanded indefinitely *in vitro* and are pluripotent, which means that they have the capacity to originate all types of tissue-specific cells of the

organism. In the post-natal organism, undifferentiated adult stem cells (ASCs) are responsible for the replacement of cells that are lost naturally or by tissue injury. They may be isolated from virtually any organ or tissue, without ethical issues as with ESCs, and since they can be obtained from the patient, they are not immunologically rejected when used for therapeutic purposes.

For cell therapy applications, one of the most promising ASC types is the mesenchymal stem cell (MSC). This review will focus on recent advances in understanding MSC biology and its applications in cell therapy.

Organ-specific stem cells

During the last few years, intensive research has focused on ASCs, showing that each organ or tissue has its own compartment of stem cells. These organ-specific stem cells are slow cycling cells responsible for cell replacement in a process that involves proliferation and differentiation[1]. The rate of replenishment by stem cells

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Melissa Camassola completed her MSc in Genetics at Universidade Federal do Rio Grande do Sul (Brazil) in 2004, and her PhD at the same Institution in 2008. She is currently a research professor at the Universidade Luterana do Brasil, leading the Laboratory of Stem Cells and Gene Therapy. Her research interests are in genetics, gene therapy and adult stem cell research. She may be contacted at Post-Graduation Program on Genetic and Molecular Diagnostics, Lutheran University of Brazil, Av. Farroupilha, 8001 - Predio 22, CEP 92425-900 – Canoas, RS, Brazil.
*E-mail: camassola@gmail.com

Pedro Chagastelles completed his MSc in Genetics at Universidade Federal do Rio Grande do Sul (Brazil) in 2006, working with cell therapy in a murine model of diabetes. He is currently concluding his PhD in genetics at the same university, participating in a Sandwich PhD programme at the Beta Cell Development and Function Group, King's College London. His research project involves the investigation of the capacity of mesenchymal stem cells to differentiate into insulin-producing cells *in vitro*.

Nance Beyer Nardi received her MSc in Genetics and Molecular Biology from Universidade Federal do Rio Grande do Sul, Brazil (1978) and her PhD in Immunology at University of London (1984). She is currently lecturer and researcher at Universidade Federal do Rio Grande do Sul and Universidade Luterana do Brazil. Her main research interests include gene therapy and mesenchymal stem cells.

will depend on the rate of cell death by apoptosis or tissue injury, which is variable for different organs or tissues. Some organs, such as the skin and intestine, have a fast renewing rate (high turnover), with all epithelial cells being replaced every 5 days in mice, for example. Some of the main characteristics of tissue-specific stem cells are presented in Table 1.

The *in vivo* behaviour of stem cells is intimately linked to their environment, or ''niche'', which gives the conditions for stem cells to be maintained in an undifferentiated state (self-renew) and to differentiate when required. This process is mediated by cell-to-cell contact, by components of extracellular matrix and soluble factors. The niche is well described for some types of ASCs, such as the haematopoietic stem cell (HSC). The post-natal niche of HSCs is the bone marrow, where MSCs play an important role[14].

Mesenchymal stem cells

MSCs are a special type of adult stem cells, initially isolated from the bone marrow[15]. These cells were first studied by Friedenstein[16] and the number of preclinical and clinical studies that employ these cells has increased exponentially in the last years[17]. Pittenger *et al.*[18] described a population of adherent cells that may be expanded in culture and could originate differentiated adipocytes, osteoblasts and chondrocytes. MSCs cells are also negative for CD14, CD34 and CD45 and positive for SH2 (CD105) and SH3 (CD73). According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy, the minimal criteria to define human MSCs are the capacity of plastic-adherence when in standard culture conditions. Cells must be positive for CD105, CD73 and CD90, and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Additionally, they must have the functional capacity to differentiate *in vitro* into osteoblasts, adipocytes and chondroblasts[19]. The name mesenchymal stem cells should only be used for cells that match these criteria, plastic adherent non-characterized cells should be named as multipotent mesenchymal stromal cells[20].

No exclusive MSC marker has been described, which makes difficult the isolation of MSCs from fresh organs and tissues. The same technical problem happens to HSCs and other tissue-specific stem cells but some markers, such as CD34 for HSCs and Stro-1 for MSCs, allow the enrichment of specific stem cells populations.

In spite of intensive investigation on the *in vitro* characterization of these cells, the biology and role of MSCs *in vivo* is still poorly

Table 1 *Characteristics of adult stem cells that include mesenchymal or MSC-like stem cells and other types of tissue-specific stem cells*

	Stem cell	Organ/niche	Surface markers	Progeny	Ref.
MSC-like stem cells	Mesenchymal stem cells	All vascularizated organs and tissues/ Perivascular niche	CD45 −, CD14 −, CD34 −, HLA − DR −, CD73 +, CD90 +, CD105 +	Adipocytes, osteoblasts, chondrocytes and myoblasts	2
	Dental pulp stem cells	Teeth	Similar to MSCs	Adipocytes, osteoblasts, chondrocytes and myoblasts	3
	Adipose-derived stem cells	Fat tissue	Similar to MSCs	Adipocytes, osteoblasts, chondrocytes and myoblasts	4
Tissue-specific stem cells	Haematopoietic stem cells	Bone marrow/ Endosteal and vascular niche	CD45 +, CD34 +, CD38 −	All hematopoietic stem cells (lymphocytes, macrophages, erythrocytes...)	5
	Liver stem cells	Liver/Canals of hearing	CK7 +, CK19 +, CD133 +, c-kit	Hepatocytes or cholangiocytes	6
	Pancreatic stem cells	Pancreatic ducts	CD133 +, CK19 +, c-met +	All islets subtypes, acinar and ductal cells	7

Table 1 *Characteristics of adult stem cells that include mesenchymal or MSC-like stem cells and other types of tissue-specific stem cells* (cont.)

	Stem cell	Organ/niche	Surface markers	Progeny	Ref
Tissue-specific stem cells	Intestinal stem cells	Intestinal crypt (+4 position)	Sox-4, sFRP5, Dcamkl1, phosph PTEN and phospho-AKT	Enterocytes, globet cells, enteroendrocrine cells and paneth cells	8
	Epidermal stem cells	Skin/basal layer	b1 integrin, a6 integrin, P63	Spinous cells, granular cells and stratum corneum	9
	Kidney stem cells	Controversial/ Bowman's capsule, glomeruli, papilla	CD133+, CD24+, Sca-1+, Lin-	Tubular phenotype, mesangial cells	10
	Cardiac stem cells	Heart	Lin-, c-kit+	Myocytes, endothelial cells and smooth muscle cells	11
	Satellite cells or muscle stem cells	Muscle/surface of muscle fibres	Pax7+	Myoblasts, myofibres	12
	Neural stem cells	Subventricular zone	Nestin, CD133+, NCAM	Neurons, oligodendrocytes, astrocytes	13

understood. Recent studies demonstrate that MSCs can be isolated from virtually all organs and tissues, suggesting that they reside in association with blood vessels in a perivascular niche[2,17]. Pericytes are cells localized on the abluminal side of blood vessels in close association with endothelial cells. These cells can receive special names depending on in which organ they are localized. A recent review of our group proposes that MSCs and pericytes can be the same cell type[17]. This affirmation is based on similarities between them, such as the presence of several surface proteins (stro-1, nestin, α-smooth muscle actin, CD44, CD90 and CD105). Speculations about the identity of MSCs suggest that they may be also closely related to conventional fibroblasts, based on shared characteristics that include phenotype, differentiation capacity, immunosuppressive properties, distribution in the organism, and growth potential[21]. Further studies need to be performed to determine if they are exactly the same cell type or are cells sharing generic properties but with a specialized function. We draw attention to the importance of characterizing the cells in basic, preclinical and clinical studies, mainly because it is possible to find more than one type of stem cells (tissue-specific and MSCs) in organs and tissues.

Transdifferentiation x paracrine effect

MSCs are currently considered as the adult stem cells with the greatest potential for therapeutic applications[22]. Mechanisms responsible for this therapeutic role are not well understood, as happens with other types of adult stem cells, and may involve differentiation or paracrine activity[23].

The embryonic origin of MSCs is uncertain but some evidence suggests that they derive from mesoangioblasts from the embryonic dorsal aorta (mesoderm germ layer), which explains their facility to differentiate *in vitro* into adipocytes, osteoblasts, chondrocytes and myocytes[17] (Figure 1). Transdifferentiation of mesenchymal stem cells (mesodermal origin) into neurons (ectodermal origin) or hepatocytes and beta cells (endodermal origin) has already been suggested, but remains highly controversial, due to the possibility of technical artefacts involved with *in vitro* culture systems[24]. Protocols for differentiating MSCs into insulin-producing cells, for example, show that differentiated cells can produce little insulin compared to a beta cell[25] or they do not differentiate completely *in vitro*[26]. Barnabé *et al*.[27] induced the differentiation of rat mesenchymal stem cells to a neuronal phenotype with a

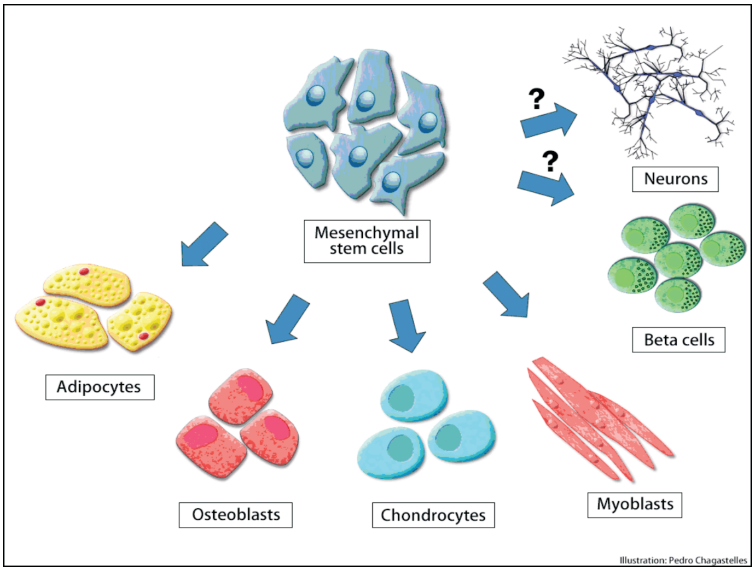

Figure 1 *Mesenchymal stem cells are currently considered the adult stem cell type of greatest plasticity, but differentiation into cells of different germinal layers is still under debate.*

combination of chemical compounds. Resulting cells showed a neuronal-like morphology as well as the expression of neuronal markers, but lack of basic functional neuronal properties. Nevertheless, several studies still aim to differentiate MSCs into several specialized cell types *in vitro*, through the combination of cytokines, growth factors and biomolecules[22]. In some cases, the differentiation process can be improved by *ex vivo* genetic manipulation with vectors expressing key transcription factors specific for each cell type[26].

MSC therapy in several preclinical disease models has shown very little *in vivo* differentiation of the transplanted cells, so that transdifferentiation is not recognized as the main mechanism that explains improvements after treatment[17,28]. On the other hand, MSCs produce and secrete a vast panel of cytokines, growth factors and chemokines, with angiogenic, immunosuppressive, anti-apoptotic and proliferative properties[29]. An example of that is hepatocyte growth factor (HGF) secreted by MSCs, that induces the proliferation of tissue-specific stem cells such as the neural stem cells and epithelial progenitor cells[7]. *In vivo*, dental pulp mesenchymal stem cells induce the proliferation and differentiation of endogenous neural stem cells when transplanted in the hippocampus

of mice[3]. Other MSC-produced factors, such as VEGF, bFGF, angiopoietin-2 and FGF4 among others that are produced by MSCs, mediate angiogenesis[29]. Most evidence, therefore, suggests the paracrine effect as the major mechanism responsible for tissue regeneration and for the success of MSCs in protocols of therapy[17].

MSCs and the culture dish

The frequency of mesenchymal stem cells in the bone marrow is very low, so that, for clinical applications, they must be expanded *in vitro* for weeks or months. This expansion process can result in undesirable effects such as the loss of ''stemness'', senescence and genetic instability[22]. Human bone marrow MSCs normally expand *in vitro* but progressively present telomere shortening and do not express hTERT transcripts or telomerase activity[30]. Cell culture, however, is generally not followed by chromosomal abnormalities, suggesting that MSCs are safe for cell therapy even after *in vitro* expansion. MSCs from other species, such as rhesus macaques, may present tetraploidization at late passages, as well as alterations in cell cycle, cell cycle checkpoint and apoptosis[31]. Murine MSCs acquire chromosomal abnormalities early in culture, can became malignant and originate tumours in mice[32].

The expansion process requires the addition of factors that induce MSC proliferation. Foetal calf serum is most generally used, but for human applications cells must be cultivated free of xenomaterials to avoid contamination with pathogens and a possible immune response against residual antigens. The alternative is to use autologous serum or a serum-free culture medium supplemented with factors that still must be well defined for expansion of MSCs for clinical application[28].

Mesenchymal stem cells and the immune system

One important property of MSCs that emerged when mechanisms responsible for their therapeutic potential were explored, is the ability to modulate the immune system. *In vitro* and *in vivo* studies have explored the mechanisms by which MSCs exert immunosuppressive effects[33]. MSCs are not immunogenic, so that they may be used in allotransplantation (same species) or even in xenotransplantations (different species) without rejection. They are able to inhibit the proliferation of subsets of T lymphocytes and B lymphocytes, as well as the differentiation, maturation and function of antigen-presenting cells. The proliferation and cytotoxicity of NK cells in

the presence of MSCs is inhibited and the generation of regulatory T cells is favoured[34]. These effects are mainly exerted by soluble factors, and MSCs are also known to express high levels of toll-like receptors 3 and 4, two important molecules that recognize virus-derived double-strand RNA and lipopolysaccharides from gram-negative bacteria, respectively. However, the presence of these antigens inhibit the capacity of MSCs to suppress $CD4^+$ T lymphocytes[35], indicating a mechanism of regulation in which MSCs starts to inhibit the immune response only after the infection is under control. MSCs have thus a central role on the modulation of immune responses, which opens a wide range of applications, mainly for autoimmune diseases and graft-versus-host disease (GVHD)[36].

In vivo function of MSCs

As proposed by our group[17], MSCs have a perivascular location and contribute to blood vessel stabilization and tissue homeostasis. This model proposes that, besides replenishing tissues with MSCs, MSCs have a more active role in the repair of focal tissue injury. In the case of tissue injury, MSCs secrete a panel of cytokines and factors which control the immune response to avoid an autoimmune process. They also act to stimulate the formation of new blood vessels, inhibiting local apoptosis and stimulating the proliferation of tissue-specific stem cells.

Therapeutic applications of MSCs

Characteristics presented by MSCs, such as their expansion potential, ease of collection, plasticity and immunosuppressive activity, make them attractive candidates for clinical cell therapy trials[37]. The cells can be administered locally or systemically, due to their ability to migrate to sites of lesion. A large number of preclinical studies have also been performed with MSCs, but results have been heterogeneous[22], possibly because of a lack of standardization of disease models, tissue culture conditions and characterization of the cells employed. Preclinical studies also differ in the number of cells employed, route and time of administration. This rapidly expanding field is represented below by considering the use of MSCs in cardiac and autoimmune diseases, as well as their potential applications in the treatment of cancer.

Cardiac diseases

Clinical stem cell therapy in cardiac diseases started with several phase I and II studies employing bone marrow mononuclear cells (BMMCs). BMMCs proved to be safe for therapy but with a modest effect in improving the cardiac function[38,39]. Some phase I and II studies are now recruiting patients for therapy of myocardial infarction, dilated cardiomyopathy and ischaemic heart disease with autologous MSCs, according to the data of the National Institutes of Health (ClinicalTrials.gov). In some cases, the studies do not specify the type of cell to be used. Only three studies have been published so far and it is too early to anticipate any results, except for the safety of the procedure[28].

Autoimmune diseases and GVHD

While the immunosuppressive effects of MSCs are clearly seen *in vitro*[40], results in animal models are more controversial. GVHD, which occurs after bone-marrow transplantation and is caused by the immune activity of the transplanted cells against host tissue, can be treated by MSCs in mice. Other studies, however, showed no effect of MSCs[41], nor that MSCs can prevent but not treat GVHD in mice[42]. Few studies have used MSCs in autoimmune models, but positive results were described for autoimmune encephalitis and autoimmune diabetes[43], while contradictory results were observed on experimental rheumatoid arthritis[44,45]. Several phase I and II studies are recruiting patients for GVHD treatment with MSCs, and results from these studies are expected to clarify the real therapeutic benefits of MSCs[46]. In September 2009, Osiris Therapeutics, Inc., Columbia, MD, announced preliminary results for two Phase III trials evaluating the use of a preparation of MSCs specially formulated for intravenous infusion (Prochymal) for the treatment of GVHD. While significant improvements in response rates in difficult-to-treat liver and gastrointestinal GVHD were observed, neither trial reached its primary endpoint. Based on these results, Osiris Therapeutics, Inc plans to apply for a broadening of the entry criteria to include patients with severe GVHD of the liver. Clearly, more preclinical studies are needed, so that the *in vivo* immunosuppressive effect of mesenchymal stem cells is known in greater detail, allowing the design of more efficient clinical trials.

Mesenchymal stem cells and cancer

Studies evaluating the therapeutic potential of MSCs for cancer are producing interesting results. Injected MSCs seem to have tropism for glioma sites, probably attracted by cytokines released by tumour cells[47]. This behaviour is of great interest, since MSCs can be modified genetically to produce anti-tumourigenic molecules that will be released in or in the vicinity of tumour sites, whereas healthy tissue remains preserved[48]. In some situations, however, MSCs can accelerate tumour growth and metastasis. The allogeneic cotransplantation of MSCs with a melanoma lineage, for instance, has been shown to favour tumour growth[49]. This effect may be explained by the immunosuppressive effect of MSCs, which prevents normal immune responses against malignant cells. Other unwanted properties are the capacity for inducing neoangiogenesis as well as the production of several other anti-apoptotic and mitogenic factors, that accelerate tumour growth. They may also have a role as tumour-associated stromal cells, contributing to tumour progression[50]. Further studies are necessary to determine the true potential of MSCs on different types of tumours.

Conclusions

MSCs have a perivascular niche in the organism, which explains their isolation from any vascular tissue. MSCs easily differentiate into osteoblasts, adipocytes, chondrocytes and myoblasts *in vitro*, but it is still unclear why this would be important for MSCs located in non-mesenchymal tissues such as the brain or liver. The immunomodulatory, anti-apoptotic and regenerative properties of MSCs suggest they have a major role in tissue homeostasis and regeneration. Preclinical studies show promising results in some disease models, but controversial results are still reported, indicating the need for further basic and preclinical investigation on their therapeutic potential. Clinical trials with MSCs are in the preliminary stages, but have already shown the safety and feasibility for different diseases.

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