Biology and clinical utilization of mesenchymal progenitor cells

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Abstract

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Within the complex cellular arrangement found in the bone marrow stroma there exists a subset of nonhematopoietic cells referred to as mesenchymal progenitor cells (MPC). These cells can be expanded *ex vivo* and induced, either *in vitro* or *in vivo*, to terminally differentiate into at least seven types of cells: osteocytes, chondrocytes, adipocytes, tenocytes, myotubes, astrocytes and hematopoietic-supporting stroma. This broad multipotentiality, the feasibility to obtain MPC from bone marrow, cord and peripheral blood and their transplantability support the impact that the use of MPC will have in clinical settings. However, a number of fundamental questions about the cellular and molecular biology of MPC still need to be resolved before these cells can be used for safe and effective cell and gene therapies intended to replace, repair or enhance the physiological function of the mesenchymal and/or hematopoietic systems.

Origin of the concept of bone marrow-derived mesenchymal progenitors

The work initiated by Friedenstein and collaborators (1) provided definitive evidence that bone marrow contains, in addition to the hematopoietic progenitors, a population of spindle-shaped clonogenic fibroblast precursor cells or fibroblast colony-forming units (CFU-F). These cells, which were defined *in vivo* as quiescent resting cells, after proper *in vitro* stimulation can enter the cell cycle and develop colonies that resemble small deposits of bone or cartilage (2). Since CFU-F exhibit a high ability for self-renewal and multipotentiality, it was speculated that these "marrow stromal stem cells" were the pre-cursors of a number of different mesenchymal cell lineages (3,4). Thus, the concept that the marrow stromal moiety was part of a wider stromal mesenchymal system in adult organisms was developed.

Data related to the number and hierarchy of cell lineages belonging to the stromal mesenchymal system, in addition to a substantial progress in the understanding of the differentiation process and the characterization of the evolving phenotypes, open perspectives for the use of these "marrow stromal stem cells" in cellular or genetic therapies for mesenchymal disorders (5,6). In this review we will highlight in a rather selective manner the current knowledge on this stromal mesenchymal system.

The term CFU-F or marrow stromal fi-

broblasts (7) has been gradually abandoned and replaced by diverse, still indistinct denominations, like marrow stromal cells (5), mesenchymal stem cells (4), or mesenchymal progenitor cells (MPC) (8). Nevertheless, in all cases reference is made to a particular adherent cell type evolving from bone marrow-derived low density mononuclear cells, cultured in a classical medium supplemented only with selected batches of fetal bovine serum. Cells thus developed, which hereafter will be referred to as MPC, display a fibroblast-like morphology, can be expanded *ex vivo* and present a potential to terminally differentiate into at least seven types of cells: osteocytes, chondrocytes, adipocytes, tenocytes, myotubes, astrocytes and hematopoietic-supporting stroma (7,9-16). We should emphasize that the denomination "marrow stromal cells" has also been used for monolayers of long-term marrow stroma or Dexter-type cultures (17). However, culture conditions, evolving phenotypes, differentiation potential and secretion products of the above cells are not analogous to those of MPC, but are in fact quite dissimilar (18,19).

Characteristics of MPC

Human MPC cultures contain a homogenous population of fibroblast-like cells which have a population doubling time of 33 h and exhibit a large (20) but variable *ex vivo* expansive potential. It has been reported that while some MPC preparations can be expanded over 15 cell doublings, others cease replicating after about 4 cell doublings (21-23). In addition, as samples are highly expanded, MPC apparently lose their multipotentiality and approach senescence and/or express apoptotic features (20,22).

Cell cycle studies on human MPC cultures have revealed the presence of a fraction (20%) of cells with a quantitative pattern of RNA and DNA typical of quiescent (G0) cells (20). These cells can be isolated by a negative selection procedure using 5-fluorouracil, which originates a population of more than 90% G0 cells, expressing the gene for ornithine decarboxylase antizyme, a marker for cellular unproliferative status. The resting condition, together with a selective immunophenotype and the absence of the expression of commitment markers in the selected cells, suggest that within cultures of MPC a fraction of mesenchymal stem-like cells subsists (Conget P, unpublished results). This finding gives experimental support to the hypothesis that a "rare" mesenchymal stem cell in the bone marrow is capable of self-renewal and differentiation into various mesenchymal lineages.

The antigenic phenotype of MPC is not unique, borrowing features of mesenchymal, endothelial, epithelial and muscle cells (9,14,20,24). Since MPC do not express typical hematopoietic lineage markers (CD14, CD34, CD45) (9,20), it has been postulated that bone marrow hosts at least two main different stem/progenitor cells which can give rise to mature hematopoietic and mesenchymal cells (5,25).

The extended cytokine expression profile of MPC, which includes several hematopoietic and nonhematopoietic growth factors, interleukins and chemokines (18,26), suggests that MPC contribute to the marrow microenvironment with inductive/regulatory signals for the development of hematopoietic cells as well as for stromal cells, including the MPC itself. The latter is sustained by recent data showing that MPC express numerous growth factor and cytokine receptors (9, and Erices A, unpublished results), suggesting that the function of these cells is under the control of autocrine or juxtacrine loops. Additional evidence for the dynamic function performed by MPC in the marrow microenvironment is given by data revealing their capacity to produce and organize a vast array of extracellular matrix molecules (19). Moreover, MPC express several counterre-

ceptors associated with matrix- and cell-to-cell adhesive interactions (9,20).

Differentiation potential of MPC

One of the first descriptions of the *in vivo* differentiation potential of MPC was the report showing that after a successful and uneventful HLA-matched marrow allograft, a dog suddenly died of respiratory failure due to extensive ossification of the lungs with multiple sites of hematopoietic engraftment (27). This study, described by the authors as "an unexpected phenomenon", was followed by several studies showing that in animal models, cultured MPC once transplanted can develop into terminally differentiated mesenchymal tissues, like bone (7,10, 28-30), cartilage (10,29,31), tendon (13,32), muscle (33), neural (16) or hematopoietic microenvironments (7). The given examples just reaffirm the broad multipotentiality of MPC, probably the adult stem/progenitor cell exhibiting the highest degree of plasticity (6,34).

Most of the studies on the *in vitro* differentiation potential of MPC, mainly into osteoblasts, chondrocytes, myotubes and hematopoietic-supporting stroma, came from the work by Caplan and colleagues (4,11,15,35). These studies have provided information regarding culture conditions, proper stimuli and methods for identification of the respective ultimate differentiated phenotype.

The molecular and cellular events associated with differentiation pathways are not well understood, but it seems that the commitment to the osteo-chondrogenic or adipogenic lineages requires the expression of Cbfa-1 or PPARγ2, respectively (36,37). Subsequent maturation along these pathways includes the expression of alkaline phosphatase, osteopontin, osteocalcin and collagen I in the osteocytic lineage; collagen II and IX in the chondrocytic lineage, and aP2, adipsin, leptin and lipoprotein lipase in the adipocytic one (9,38). Thus, analyses at the gene expression level (RT-PCR) have shown that MPC differentiate *in vitro,* according to the stimuli applied, into the desired lineage but not into cells expressing multiple lineages (9).

Although diagrams for a hierarchy of MPC progenitors evolving from a putative mesenchymal stem cell have been published (6,39), data explaining how lineage choices and transcriptional specificities are achieved and how these account for the extraordinary multipotency of mesenchymal progenitor cells are lacking. It will be challenging for investigators in the field to fill in the gaps on these issues.

Sources of MPC

Recent data have shown that, in addition to adult bone marrow, umbilical cord blood is also a source of MPC (40). These cells exhibit an immunophenotype, a population of quiescent cells and a differentiation potential similar to that of marrow-derived MPC. The observation that the content of MPC is higher in preterm than in term cord blood, a trend also observed for hematopoietic progenitors (41), suggests that hematopoietic and mesenchymal progenitors travel early during development, probably from fetal hematopoietic sites to the newly formed bone marrow via cord blood (42).

Whether MPC circulate in peripheral blood is an open issue. In the murine model, CFU-F circulate in blood and represent a stromal cell population which can migrate into hematopoietic organs (43). In humans, cells with the characteristics of mesenchymal progenitors were detected in growth factor-mobilized peripheral blood stem cells harvested from breast cancer patients, but not in the blood from normal donors (44). However, under similar but not identical experimental conditions, the presence of circulating MPC has not been confirmed by other groups (45,46).

Transplantation of MPC

The envisioned routes of MPC delivery are either direct loading (injection or implants) into the damaged organ or systemic infusion. In the former case, it has been proposed that MPC will augment local repair or regeneration of bone (28-30), cartilage (47) or tendon (32). With respect to systemic infusion, MPC should home into the damaged tissue and restart their developmental program. Thus, MPC will improve target tissue function (48,33) or increase marrow microenvironment support to facilitate engraftment by hematopoietic stem cells (6,49).

Despite the profuse information on the origin (host or donor) of stromal cells after allogeneic transplantation, the issue is still open because of contradictory data. Thus, it has been reported that after successful allogeneic bone marrow transplantation (considered as a source of hematopoietic and mesenchymal progenitors), MPC isolated at different time intervals after transplantation exhibit cellular and molecular features that correspond either to the host (50-52) or to the donor (48,53,54). The nature of this conflict may arise from several determinants, among them the methods used to type MPC, the procedure followed to harvest the marrow (21,55), the low frequency of MPC in marrow harvests (2-5 MPC per 1×10^6 mononuclear cells) (50), and/or the condition (steady state *vs* post chemo- or radiotherapy) of the marrow from which MPC were prepared (22,52) and to which MPC were transplanted.

An additional explanation for the discrepancy about the marrow transplantation capacity of MPC may arise from the observation that the number of mesenchymal stem-like cells among different cultures is low and variable (20, and Conget P, unpublished results). Based on data for the hematopoietic and muscle system (56,57), one can speculate that quiescent and cycling MPC present in the graft will contribute in a different way to stromal repopulation after transplantation. Mesenchymal stem-like cells, after homing to the marrow space will self-renew and thereby sustain long-term mesengenesis. In turn, cycling MPC which will probably also home to other mesenchymal tissues due to their committed condition, will only contribute to short-term mesengenesis. Whatever the case, there are still many open questions concerning the transplantability of MPC, either as isolated cells, after *ex vivo* expansion, or as whole cells with hematopoietic progenitors (55).

Clinical trials using MPC

Given the promising features of adult stem cells for the development of new cell therapies (6,34), researchers in the field of MPC have pursued a broad range of lines of investigation to stimulate their therapeutic utilization.

The first clinical trials reported have revealed that systemic infusion of *ex vivo* expanded autologous MPC is feasible and safe in the short-term (49,58). However, there is yet no conclusive evidence to support the contention that transplanted MPC may have a positive impact on the management of lymphohematopoietic or cancer patients (49). On the other hand, it has been demonstrated that allogeneic bone marrow transplantation in children with osteogenesis imperfecta results in impressive histological changes in trabecular bone which indicate new dense bone formation (59). In addition, increased growth rate and reduced frequencies of bone fracture were also observed. These changes, detected 3 months after marrow transplantation, were associated with the engraftment of functional MPC from the transplanted marrow (25). Surprisingly, recent reports have documented that following bone marrow transplantation, short-term changes in bone mineral metabolism caused a rapid impairment of bone formation and an increase in bone resorption (60).

Conclusions and future directions

The last five years have been the scene of a substantial improvement in our understanding of the biology and the potential clinical utilization of adult MPC. Although many aspects related to the properties of these cells are well established, information dealing with the existence of a hierarchy of mesenchymal precursors (including the mesenchymal stem cell itself) and their properties still remains obscure. However, this lack of information has not been an obstacle in terms of the therapeutic utilization of these cells.

MPC represent an attractive therapeutic option, both in the context of cellular and gene therapy strategies for a wide range of clinical applications. Future clinical trials should be focused on at least two main issues: as an integral part of the marrow microenvironment, MPC transplantation alone or in conjunction with hematopoietic progenitors would facilitate the engraftment of the hematopoietic stem cell after myeloablative therapy. Also, they might replace chemotherapy- or disease associated-damaged stroma or modulate graft versus host disease; transplantation of MPC, as precursors of several mesenchymal lineages, is envisioned as the proper therapy to attenuate or correct disorders of mesenchymal tissues, like osteogenesis imperfecta, osteoporosis, osteoarthrosis, meniscectomy, muscular dystrophy, etc.

To improve the latter, several studies have shown the feasibility of adeno- or retroviral-mediated gene transfer of reporter or therapeutic genes into MPC (8,61,62). For the near future, we anticipate a rapid closure of many gaps in our knowledge of the biology of MPC, which may facilitate the development of phase II and III clinical trials for new therapeutic alternatives using MPC. Thus, as recently proposed, MPC are "no longer second class marrow citizens" as compared with hematopoietic progenitors, the paradigm of bone marrow cells (25).

Acknowledgments

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