

Preface

The discovery of mesenchymal stem cells is credited with Alexander Friedenstein and associates, who over 40 years ago demonstrated that pieces of bone marrow transplanted under the renal capsule of mice formed a heterotopic osseous tissue that was self-maintaining, self-renewing, and capable of supporting host cell hematopoiesis. Furthermore, Friedenstein showed that the osseous-forming activity of bone marrow was contained within the fibroblastoid cell fraction isolated by preferential attachment to tissue culture plastic. These findings confirmed that bone marrow contained separable stem cell populations capable of generating hematopoietic and connective tissue cell lineages. These studies also demonstrated that marrow-derived, plastic adherent fibroblastic (stromal) cells were capable of supporting the growth and differentiation of various hematopoietic cell types. These cells were then used as feeder layers to establish long-term bone marrow cultures *in vitro*, which fostered a wealth of new knowledge regarding the molecular mechanisms regulating hematopoiesis.

In the decades following Friedenstein's seminal publications, various groups labored to delineate the biological nature and differentiation potential of plastic adherent cells from bone marrow. These efforts revealed much information about their cell surface phenotype, proliferative and differentiation potential and culminated in the demonstration that clonally derived murine and human populations were multipotent, capable of differentiating into adipocytes, chondrocytes, osteoblasts, and hematopoiesis-supporting stromal cells. The latter findings confirmed the existence of a stem cell in marrow capable of generating most connective tissue cell types. Consequently, the marrow-derived, plastic adherent cells first referred to as colony-forming unit fibroblast (CFU-F) by Friedenstein, then in the hematological literature as marrow stromal cells, subsequently became known as mesenchymal stem cells. Recently, a committee from the International Society of Cell Therapy has adopted the term multipotent mesenchymal stromal cells (MSCs) to define these cells owing to the fact that a definitive description of the bona fide mesenchymal stem cell and the molecular mechanisms that regulate its self-renewal versus differentiation remain forthcoming. The literature has been confused by the frequency with which the different names for essentially the same cells have been used (*see* Fig. 1). In this compendium, the terms CFU-F, marrow stromal cell, mesenchymal stem cell, and multipotent mesenchymal stromal cell

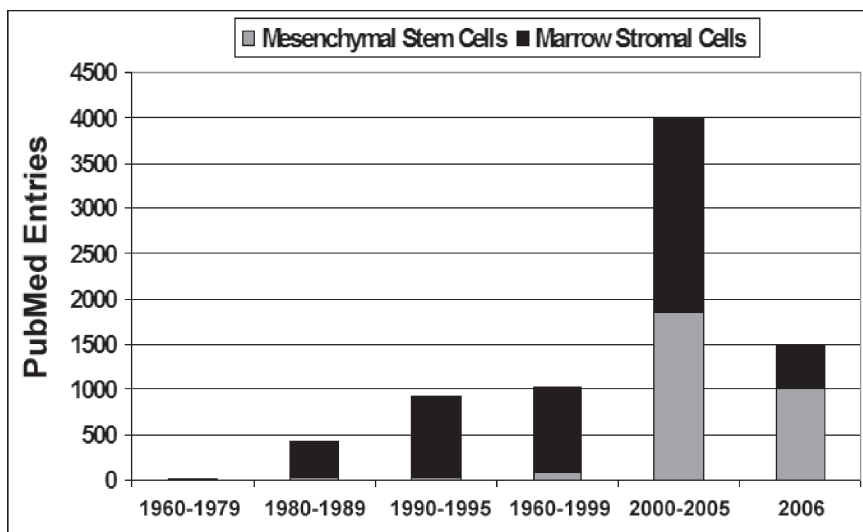


Fig. 1 Illustrated is the number of citations found in the PubMed database that contain the phrase “marrow stromal cells” or “mesenchymal stem cells” in their title or abstract over various time periods.

are deemed equivalent and herein will refer to the plastic adherent, fibroblastoid cells from marrow that are defined functionally by their capacity to undergo multi-lineage differentiation into connective tissue cell lineages.

In recent years MSCs have garnered much attention owing to their broad therapeutic efficacy. Initially, MSC administration to children afflicted with osteogenesis imperfecta was found to have a significant positive impact by reducing the severity of the disease. Promising results were subsequently reported using MSCs or related cells from bone marrow in the treatment of Hurler’s syndrome, metachromatic leukodystrophy, graft versus/host disease and to enhance engraftment of heterologous bone marrow transplants. Most recently, MSCs have been shown to afford a therapeutic benefit in the treatment of myocardial infarction, stroke, lung diseases, spinal cord injury, and other neurological disorders. These results, together with the fact that MSCs can be readily isolated from small volume bone marrow aspirates, expanded to large numbers *ex vivo* and engineered genetically have made them extremely attractive as therapeutic cellular vectors.

Despite these advances, it has been difficult to assess the overall therapeutic use of MSCs owing to conflicting reports in the literature regarding their engraftment levels in tissues *in vivo*, their overall differentiation potential *in vitro* and *in vivo*, as well as their therapeutic efficacy in disease models. Although some of these discrepancies are related to limitations associated with experimental methodologies, critical differences in the preparation and expansion of donor cells used for the experiments certainly contribute to this problem, as well. Consequently, the

necessity of developing standardized methods to isolate, phenotype, and evaluate the quality of MSCs is ever increasing. Accordingly, the following compendium provides detailed methodologies for the isolation and characterization of human and rodent MSCs contributed by a group of assembled leaders in the field.

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Mesenchymal Stem Cells

Methods and Protocols

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2008, XVI, 192 p., Hardcover

ISBN: 978-1-58829-771-6

A product of Humana Press