Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs)

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Abstract

In this review, we focus on the adult stem/progenitor cells that were initially isolated from bone marrow and first referred to as colony forming units-fibroblastic, then as marrow stromal cells and subsequently as either mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs). The current interest in MSCs and similar cells from other tissues is reflected in over 10,000 citations in PubMed at the time of this writing with 5 to 10 new publications per day. It is also reflected in over 100 registered clinical trials with MSCs or related cells (http://www.clinicaltrials.gov). As a guide to the vast literature, this review will attempt to summarize many of the publications in terms of three paradigms that have directed much of the work: an initial paradigm that the primary role of the cells was to form niches for haematopoietic stem cells (paradigm I); a second paradigm that the cells repaired tissues by engraftment and differentiation (paradigm II); and the more recent paradigm that MSCs engage in cross-talk with injured tissues and thereby generate microenvironments or ‘quasi-niches’ that enhance the repair tissues (paradigm III).

Keywords: mesenchymal stem cells • multipotent mesenchymal stromal cells • bone marrow • anti-inflammatory • anti-apoptotic • TSG-6 • STC-1

Paradigm I: the haematopoietic niche

Early attempts to culture bone marrow revealed that a small fraction of the cells that adhered to culture dishes were not haematopoietic precursors (Fig. 1). Some investigators were struck by the morphological similarity of the non-haematopoietic cells to the spindle-shaped cells that formed the stroma of marrow [1–4]. Therefore, they developed the paradigm that the cells formed niches for the propagation of haematopoietic stem cells. The paradigm proved extremely useful in that the confluent cultures of mesenchymal stromal cells (MSCs) were found to be effective feeder layers for the culture of haematopoietic stem cells [5, 6]. The niche role of MSCs was directly demonstrated by the observation that islands of haematopoiesis were formed within ceramic cubes that were seeded with human MSCs and then inserted under the skin of immunodeficient mice [7]. Also, the niche role of MSCs was indirectly supported by clinical trials in which the cells were shown to hasten the recovery of the haematopoietic system after bone marrow transplants [8].

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Paradigm II: engraftment/differentiation

Early investigators studying cultures of bone marrow were impressed with the facility with which the adherent, spindle-shaped cells differentiated into distinct cellular phenotypes. In particular, Friedenstein and others [1] demonstrated that the cells readily became mineralizing cells or chondrocytes both in culture and after implantation in diffusion chambers in vivo. These observations suggested the paradigm that MSCs might repair injured tissues by engraftment and differentiation (Fig. 1). The paradigm had broad implications for medical therapies in part because of the ease with which the cells could be isolated from a small sample of human bone marrow and then rapidly expanded in culture through 30 or more population doublings [9–11].

Early observations on engraftment and differentiation

Repair by paradigm II was supported by early observations that local administrations of MSCs improved bone repair [12]. The potential therapeutic implications of the paradigm were expanded by the observation that after systemic infusions of MSCs containing a mutated human gene into irradiated young mice, the mutated gene was detected in multiple tissues of the mice [13]. Also, further support for the therapeutic potentials was provided by the observation that infusions of MSCs from wild-type mice produced small but significant improvements in the bones of a transgenic mouse model for osteogenesis imperfecta [14]. The potential therapeutic implications were expanded still further by the observation that, after BrdU-labelled MSCs were injected into the cerebral ventricles of newborn mice, the cells migrated throughout the brain, and a few of the cells became astrocytes [15].

These early observations prompted a clinical trial in which children with severe osteogenesis imperfecta first received bone marrow transplants from a haplotype-matched normal donor and then were treated, several years later, with intravenous infusions of a large number of MSCs from the same donors [16]. The therapy produced a transient but significant improvement in the clinical course of the children. Most importantly, there was only one adverse event: one of the children developed a mild allergic reaction to foetal calf serum in which the MSCs were expanded. The results were followed by a clinical trial in which administration of MSCs produced encouraging results in children with severe lysosomal storage diseases [17]. These initial observations raised the possibility that paradigm II might provide new therapies for a broad spectrum of human diseases.

Technical challenges in testing paradigm II

The early efforts to test the paradigm encountered a series of technical challenges: (1) No endogenous markers for MSCs were available that could be used to track the cells in vivo [18]. Exogenous markers such as dyes or transduced genes were employed instead, but most produced unexpected artefacts [19–21]. (2) Only a small number of antibodies and other markers were available to follow differentiation of the cells in vivo. Also, the microscopes and algorithms to overcome some of the artefacts of immunohistochemistry were not commonly available. (3) Species differences in MSCs created a significant experimental barrier. Cultures of human MSCs were relatively easy to purify from haematopoietic precursors by simply re-plateing the cells. Cultures
of mouse MSCs remained contaminated by haematopoietic precursors through several passages. Also, as was observed much earlier with mouse fibroblasts [22], cultures of mouse MSCs expanded slowly until they underwent ‘crisis’ during which a few cells were transformed and then expanded rapidly [23]. Rat MSCs initially resembled human MSCs but at a later stage also underwent crisis and transformation [24, 25]. (4) MSCs were not readily transplanted into marrow ablated mice and therefore presented a further limitation in the use of transgenic mice. (5) Most importantly, tissue repair is a highly complex biological process that varies with the type of injury and the tissue injured [26]. Also, there are marked species differences in inflammatory and immune responses [27] and as a result many experimental animals, especially rodents, repair tissues much more efficiently than human beings. In effect, there were several serious barriers to definitive experiments to test paradigm II.

The impetus to test the paradigm II in clinical trials

Despite these technical challenges, there continues to be great interest in testing the medical implications inherent in paradigm II. The paradigm has been pursued against the history that discoveries of new therapies in medicine have rarely been linear processes. Initial tests of a potential therapy in vitro are rarely as convincing as one would like, because of the limitations of experiments with purified molecular components and the artefacts inherent in culturing cells. The data from animal experiments are usually even more limited because of the difficulty of mimicking human diseases. The history of medicine is replete with examples of therapies that failed to be developed or whose beneficial effects were not understood until after they were first tested in patients [28]. The examples include discovery of the anti-thrombotic effects of aspirin [29, 30], the need of HLA typing in bone marrow transplants [31], the revised rationale and design of bisphosphonates for therapy of bone diseases [32] and the failure of sildenafl (Viagra) as a therapy for angina despite the Nobel prize research that led to its development [33, 34] (see Supporting Information).

Tests of the paradigm II with local administrations

Engraftment and differentiation of MSCs, as predicted by paradigm II, were seen in several settings. In models for bone and cartilage defects, a series of reports demonstrated that direct implantation of MSCs themselves or MSCs embedded in scaffolds enhanced repair [35–38]. There is a consensus that some of the administered cells differentiated into osteoblasts or chondrocytes. However, most reports indicated the MSCs disappeared in several weeks [36, 39], and most of the differentiated cells seen in long-term grafts are host cells, at least in part because of the normal turnover of the tissues.

In models of cardiac defects, several reports indicated that locally implanted MSCs engrafted and differentiated into cardiomyocytes [40, 41]. However, it has not been conclusively established that locally administered MSCs provide a sufficient number of fully integrated cardiomyocytes to account for the improvements in ventricular function observed in many experiments [42].

In the central nervous system, some experiments indicated that MSCs injected into the ventricles of embryos or of newborn pups migrated throughout the brain and differentiated as the organ developed [15, 43, 44]. In one series of experiments, quantitative PCR assays indicated that the number of MSCs or MSC-derived cells increased as much as 30-fold in a few days after male MSCs were injected into the ventricles of newborn female mice [43]. The possibility of neural differentiation was supported by the observation that some preparations of MSCs differentiated in culture into dopaminergic-like neurons with the appropriate electrophysiological properties [45]. However, it was difficult to establish differentiation of MSCs into functional neural cells in vivo [46, 47].

In contrast to transplants into embryonic brains, very few MSCs injected into the brains of adult rodents survived more than 1 or 2 weeks [21, 48, 49]. Surprisingly, the rate of disappearance was about the same with human MSCs injected into the hippocampi of both immunodeficient and wild-type mice [49].

In models for spinal cord injury, local administration of MSCs produced improved motor function but few, if any, of the cells engrafted for prolonged periods or differentiated into neural cells [50, 51]. One initial impression was that the cells formed a scaffold for regeneration of nerve tracts in the cord [51]. A recent study suggested that the therapeutic benefits were explained by anti-inflammatory effects of the cells [52].

Tests of paradigm II with systemic infusion

Tests of paradigm II with systemic infusions of the cells proved problematic. Numerous reports described functional improvements after systemic infusions of MSCs in models for human diseases that included osteogenesis imperfecta [53]; stroke [54]; myocardial infarction [55]; acute kidney injury [56] and diabetes [57, 58]. The initial interpretations of the data were based on paradigm II and assumed that the cells had homed to injured tissues, engrafted for prolonged periods or differentiated into neural cells 1 or 2 weeks [21, 48, 49]. Surprisingly, the rate of disappearance was about the same with human MSCs injected into the hippocampi of both immunodeficient and wild-type mice [49].

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To explore the paradox, we recently employed quantitative PCR assays for human MSCs infused into mice [62], a strategy introduced earlier by Phinney and associates for tracking MSCs infused into the brain [43]. (Previous data developed from gel-based PCR assays probably overestimated engraftment of MSCs after systemic infusion [13].) An improved protocol for quantitative PCR
assay of human Alu sequences demonstrated that after i.v. infusion of the human MSCs, essentially all of the cells were cleared from the circulation within 5 min. [62]. Most of the human cells were recovered in the lungs. The cells in the lungs disappeared with a half-life of about 24 hrs but only trace amounts were recovered in the six other tissues that were assayed. Therefore, the results questioned whether paradigm II could account for the functional improvement observed after intravenous infusions of MSCs in animal models for diseases of distal organs. In addition, paradigm II could not account for reports that conditioned medium from cultures of MSCs was as effective in some disease models as the cells themselves [63–65].

Paradigm III: transient ‘quasi-niches’

The accumulating evidence that MSCs could repair injured tissues without significant engraftment and differentiation called for a new paradigm that required re-examination of some of the early observations on cultures of the cells and more detailed examination of their effects in vivo (Fig. 1).

Unusual features of MSCs in culture

The early observations that confluent and non-propagating MSCs provided effective feeder layers for cultures of haematopoietic cells were explained in part by the cells secreting paracrine factors [5, 6, 66, 67]. However, the effectiveness of MSCs as feeder layers was not entirely explained by secretion of soluble factors; cell to cell contact was also required for reasons that were not apparent [5, 6].

Unusual features of MSCs in culture were also apparent from observing the cells after they were plated at clonal densities. The cells expanded as single-cell derived colonies but the properties of the cells changed as the colonies expanded. In the many of the colonies that formed, distinct inner and outer regions were apparent. The outer regions consisted of rapidly self-renewing cells and the inner regions consisted of slowly replicating cells that were partially differentiated [68]. Moreover, the cells displayed a remarkable plasticity in that the cells from both the inner and outer regions were involved in immune responses to the ischemic environment. Assays of RNA from the hippocampus with human-specific mRNA/cDNA microarrays demonstrated that in the ischemic injured brain, the human MSCs increased expression of genes that modulated immune and inflammatory responses. Assays of the same RNA on mouse-specific microarrays demonstrated that the presence of the human MSCs modulated expression of mouse genes involved in immune responses to the ischemic environment.

A similar example of cross-talk was obtained by using species-specific mRNA/cDNA microarrays to survey the lungs of mice a few hours after intravenous infusions of human MSCs [62]. By producing microemboli, the human cells altered expression of hundreds of mouse genes in the lung. At the same time, signals from the mouse cells altered expression of hundreds of genes in the human MSCs. In parallel with these observations, reports from several laboratories demonstrated that the expression of potentially therapeutic cytokines was markedly increased by exposing MSCs to cytokines typically released by injured tissues [70, 71].

Modulation of inflammation in paradigm III

The experiments in which human MSCs were infused intravenously into mice with myocardial infarcts provided a clue to how they enhanced tissue repair. One of the most interesting genes up-regulated in human MSCs that were trapped in the lung after intravenous infusion [62] was tumour necrosis factor (TNF)-α-stimulated gene/protein-6 (TSG-6) [72, 73]. Extensive previous research demonstrated that TSG-6 had remarkable anti-inflammatory properties in a number of experimental settings, including in both wild-type and transgenic mice [72, 73]. Experiments with recombinant TSG-6 and siRNAs demonstrated that the secretion of TSG-6 by MSCs trapped in the lung largely accounted for previous reports that intravenously administered MSCs improved mice with myocardial infarcts [55, 74–76]. TSG-6 decreased...
activation of the inflammatory network of proteases in the heart and decreased monocyte and granulocyte infiltration. The TSG-6 thereby decreased the damage to cardiomyocytes and the size of the myocardial scar that subsequently formed (Fig. 2).

Modulation of apoptosis in paradigm III

Several reports indicated that one of the potential therapeutic effects of MSCs was to decrease apoptosis [77, 78]. Co-culture experiments demonstrated that MSCs decreased apoptosis in two model systems in part by being activated to express stanniocalcin-1 (STC-1), a calcium regulatory protein [79]. The effects of STC-1 on apoptosis were apparently explained by its uncoupling of oxidative phosphorylation and suppression of reactive oxygen species [80]. Suppression reactive oxygen species also explains the recent observation the STC-1 has anti-inflammatory properties [81].

Modulation of immune reactions

Preliminary observations made in clinical trials to improve bone marrow transplants with MSCs provided an unexpected observation: In a few patients, the MSCs improved the effects of graft-versus-host disease [82]. These and related observations led to experiments that demonstrated intravenous infusions of MSCs reduced neurological deficits in the experimental autoimmune encephalitis (EAE) model for multiple sclerosis (see [83]). The findings spurred extensive efforts to define the mechanisms whereby MSCs modulated the immune system. The results have provided several different scenarios. Here we will focus on four recent accounts. (For more complete reviews, see [83, 84].)

Shi and associates [70] demonstrated that the immunosuppressive effects of murine MSCs were triggered by the cells being stimulated by interferon-γ together with any one of three other pro-inflammatory cytokines (TNF-α, IL-1α or IL-1β). The stimulated MSCs expressed several cytokines and inducible nitric oxide synthase. The chemokines attracted T cells to the MSCs and then the T cells were suppressed by nitric oxide from the MSCs. They subsequently found a marked species difference in that human and monkey MSCs did not synthesize nitric oxide under similar conditions. Instead, the MSCs suppressed T cells by secreting indoleamine 2,3-dioxygenase that depletes tryptophan in the medium or generated toxic concentrations of kynurenine and other metabolites to suppress T cells [85].

Galipeau and associates [86] examined the effects of MSCs on activated CD4+ T cells in the EAE model for multiple sclerosis. They found that the MSCs inhibited activation of the T cells by secreting both CCL2 (monocyte chemotactic protein-1 or MCP-1) and matrix metalloproteinases-9 that cleaved the CCL2 into an antagonistic derivative. The role of the soluble factors was confirmed by the demonstration that conditioned medium from MSCs inhibited activation of CD4+ T cells from EAE mice and that the effects of MSCs were not observed in CCL2−/− EAE mice. The same laboratory also demonstrated that MSCs can stimulate immune and inflammatory responses. They found that MSCs can cross-present exogenous antigen and induce an effective CD8+ T-cell immune response [87]. They can also be activated through Toll-like receptors to recruit inflammatory and immune cells [71].

Mahon and associates [88] suggested that MSCs might exert their immune regulatory effects by enhancing T regulatory cells. They demonstrated that allogeneic MSC induced expression in CD4+ T cells of forhead box P3+ and CD25+, both markers of T regulatory cells. Their results supported a sequential process in which a first step required direct contact between MSCs and CD4+ T cells followed by secretion of transforming growth factor-β1 and prostaglandin E2 by the MSCs to drive differentiation of T cells to T regulator cells.

Uccelli et al. [83] offered a more pleiotropic account of the effects of MSCs on the immune system. They suggested that
MSCs produced a variety of effects such as (1) decreased proliferation, cytotoxicity and cytokine production by NK cells; (2) impaired maturation and antigen presentation by dendritic cells; (3) decreased proliferation of T cells and impaired T helper cells and (4) decreased proliferation and antibody production by B cells.

At the moment, it is not clear which of the proposals best accounts for the immune modulatory effects of MSCs in vivo.

Paradigm III and the similarities to paradigm I

The experiments in which MSCs enhance tissue repair without significant engraftment suggest that there is a complex series of interactions between the MSCs and the injured tissues. One of the key interactions is a sequence in which TNF-α and other signals from injured tissues activate the MSCs to secrete TSG-6, STC-1 and probably other soluble factors that decrease the production of TNF-α and other inflammatory signals from the injured tissues (Fig. 3). In effect, the MSCs introduce a negative feedback loop into excessive responses by tissues that frequently occur in injuries not accompanied by invading organisms. Such excessive inflammatory and immune responses are now recognized to contribute to the pathoetiology of many diseases, including diabetes and atherosclerosis [89, 90]. Secretion of soluble factors probably explains the therapeutic effects of intravenous infusion of MSCs or conditioned medium from MSC cultures in some animal models. However, some of the therapeutic effects, such as in models for immune diseases, may require direct cell-to-cell contact and transfer of components such as mitochondria.

Conclusions/perspectives

Our knowledge of MSCs has evolved largely by serendipity, beginning with the first efforts to culture cells from bone marrow. Although our knowledge continues to expand at a rapid pace, a number of important questions still need to be addressed. Some examples include:

Why is administration of MSCs beneficial?

Bone marrow, fat and many other tissues contain MSCs or MSC-like cells. Therefore, it is not apparent why adequate numbers are not normally mobilized in response to tissue injury. One possibility is that the isolation of the cells from tissues or their expansion in culture may activate therapeutic properties of the cells that are otherwise latent. Another is that the normal mechanisms for mobilizing MSCs are simply not adequate to modulate the excessive inflammatory and immune responses to sterile tissue injuries.

Better assays for the potency of MSCs?

A major barrier to progress in the field is lack of an in vivo potency assay for MSCs. What is needed is an assay equivalent to the marrow ablated mouse that was key to essentially all the progress in the study of haematopoietic stem cells. Data on the transcriptomes or proteomes of cultured MSC are not adequate since they are simply snapshot pictures of the cells. Instead, what is needed is an assay of the potential of MSCs to respond to environmental factors such as signals from injured tissues. Unfortunately, current in vitro assays of differentiation or clonogenicity continue to disappoint. Given the multiple modes of action of MSCs, a battery of in vivo potency assays may be required.

Are MSCs pericytes?

Recent reports have provided convincing data for earlier suggestions (see [9]) that MSCs share many of the features of pericytes [91–93]; cells that have fascinated investigators since they were first described by Rouget in 1873 (see [94]). The similarities between MSCs and pericytes are impressive, including the sharing of several epitopes and the ability of pericytes to differentiate into multiple cellular phenotypes such as fibroblasts, osteoblasts, adipocytes, chondrocytes and endothelial cells. However, the overlap in properties is not complete. For example, pericytes from different vessels vary but most display contractility and myogenic
properties not observed with isolated MSCs. Also, pericytes propagate much more slowly than MSCs, *i.e.* initial population doubling rates as slow as 162 hrs [92] versus 12 to 20 hrs for MSCs. Therefore, pericytes and MSCs clearly have similar but perhaps not identical properties.

**Therapies with recombinant proteins?**

Recent observations suggest that therapies with some of the proteins produced by MSCs could replace therapies with the cells themselves. Use of the proteins has many attractions, but MSCs may provide major advantages in many situations by their responsiveness to the particular injury and their ability to deliver factors in high local concentrations. Also, as suggested by paradigm III, some of the therapeutic benefits of MSCs may require cell-to-cell contact for transfer of vesicles or other components such as mitochondria [95] that have not yet been defined.

**Additional questions in developing therapies with MSCs**

A number of additional questions need to be resolved to develop therapies with MSCs. Although no significant adverse events have been reported from clinical trials to date, all interventional therapies have some inherent risks and questions about the potential risks of therapies with MSCs must be carefully weighed against the potential benefits to patients. One question about the potential risks is whether MSCs, like embryonic stem cells or induced pluripotent stem cells, can cause tumours and malignancies [96]. The risk cannot be ignored, particularly since MSCs were observed to enhance the growth of some tumours [97]. However, MSCs in culture differ from embryonic stem cells and induced pluripotent cells in that they are not immortal cells and undergo senescence when expanded in culture. (A recent report indicated that a previous observation of malignant transformation of human MSCs during expansion in culture was explained by contamination of the cultures by small numbers of malignant cells [98].) Another question still under debate is whether autologous MSCs should be used or whether therapies with heterologous MSCs from ‘universal donors’ can be employed, a strategy currently embraced by several biotech companies. We all await the data from carefully conducted clinical trials and from additional basic research to resolve these and other remaining questions about MSCs.

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**Conflict of interest**

The authors confirm that there are no conflicts of interest.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Examples of Medical Therapies Developed after First Trials in Patients.**

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**References**

1. Owen M, Friedenstein AJ. Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found Symp. 1988; 136: 42–60.
2. Allen TD, Dexter TM. Long term bone marrow cultures: an ultrastructural review. Scan Electron Microsc. 1983; 4: 1851–66.
3. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991; 9: 641–50.
4. Dominici M, Le Blanc K, Mueller I, *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8: 315–7.
5. Eaves CJ, Cashman JD, Sutherland HJ, *et al.* Molecular analysis of primitive hematopoietic cell proliferation control mechanisms. Ann NY Acad Sci. 1991; 628: 298–306.
6. Whetton AD, Dexter TM. Influence of growth factors and substrates on differentiation of haematopoietic stem cells. Curr Opin Cell Biol. 1993; 5: 1044–9.
7. Sacchetti B, Funari A, Michienzi S, *et al.* Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell. 2008; 131: 324–36.
8. Koç ON, Gerson SL, Cooper BW, *et al.* Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol. 2000; 18: 307–16.
9. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissue. Science. 1997; 276: 71–4.
10. Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284: 143–7.
26. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA. 1999; 96: 10711–6.

27. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med. 1999; 5: 309–13.

28. Koç ON, Peters C, Aubourg P, et al. Bone marrow-derived mesenchymal stem cells remain host-derived despite successful hematopoietic engraftment after allogeneic transplantation in patients with lysisosomal and peroxisomal storage diseases. Exp Hematol. 2000; 27: 1675–81.

29. Lee RH, Hsu SC, Munoz J, et al. A subset of human rapidly self-renewing marrow stromal cells preferentially engraft in mice. Blood. 2006; 107: 2153–61.

30. Krause DS. Bone marrow-derived lung epithelial cells. Proc Am Thorac Soc. 2008; 5: 699–702.

31. Kuhn HG, Cooper-Kuhn CM. Bromo-deoxyuridine and the detection of neurogenesis. Curr Pharm Biotechnol. 2007; 8: 127–31.

32. Coyne TM, Marcus AJ, Woodbury D, et al. Marrow stromal cells transplanted to the adult brain are rejected by an inflammatory response and transfer donor labels to host neurons and glia. Stem Cells. 2006; 24: 2483–92.

33. Rubin H. Multistage carcinogenesis in cell culture. Dev Biol. 2001; 106: 61–6.

34. Tolar J, Nauta AJ, Osborn MJ, et al. Sarcoma derived from cultured mesenchymal stem cells. Stem Cells. 2007; 25: 371–9.

35. Foudah D, Redaelli S, Donzelli E, et al. Monitoring the genomic stability of in vitro cultured rat bone-marrow-derived mesenchymal stem cells. Chromosome Res. 2009; 17: 1025–39.

36. Furlani D, Li W, Pittermann E, et al. A transformed cell population derived from cultured mesenchymal stem cells has no functional effect after transplantation into the injured heart. Cell Transplant. 2008; 18: 319–31.

37. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008; 8: 349–61.

38. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2008; 172: 2731–8.

39. Ban TA. The role of serendipity in drug discovery. Dialogues Clin Neurosci. 2006; 8: 335–44.

40. Morris CDW. Acetyl salicylic acid and platelet sickness. Lancet. 1967: 289: 279–80.

41. Patrone C, Rocca B. Aspirin, 110 years later. J Thromb Haemost. 2009; 7: 258–61.

42. Thomas ED, Blume KG. Historical markers in the development of allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 1999; 5: 341–6.

43. Fleisch H. Development of bisphosphonates. Breast Cancer Res. 2002; 4: 30–4.

44. Bryan NS, Bian K, Murad F. Discovery of the nitrile oxide signaling pathway and targets for drug development. Front Biosci. 2009; 14: 1–18.

45. Ghofrani HA, Osterloh IH, Grimminger F. Sildenafil: from angina to erectile dysfunction. Dialogues Clin Neurosci. 2006; 8: 172: 2731–8.

46. McBride C, Gaupp D, Phinney DG. Quantifying levels of transplanted murine and human mesenchymal stem cells in vivo by real-time PCR. Cytotherapy. 2003; 5: 7–18.

47. Mucó-Elías G, Marcus AJ, Coyne TM, et al. Adult bone marrow stromal cells in the embryonic brain: grafting, migration, differentiation, and long-term survival. J Neurosci. 2004; 24: 4585–95.

48. Tatar VM, D’ippolito G, DiBiase S, et al. Neurotrophin-directed differentiation of human adult marrow stromal cells to dopaminergic-like neurons. Bone. 2007; 40: 360–73.

49. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair – current views. Stem Cells. 2007; 25: 2896–902.

50. Parr AM, Tator CH, Keating A. Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant. 2007; 40: 609–19.

51. Munoz JR, Stoutenger BR, Robinson AP, et al. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci USA. 2005; 102: 18171–6.

52. Ohtaki H, Yostalo JH, Foraker JE, et al. Stem/progenitor cells from bone marrow decrease neuronal death in global...
ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci USA. 2008; 105: 14638–43.

50. Chopp M, Zhang XH, Li Y, et al. Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. Neuronreport. 2000; 11: 3001–5.

51. Hofstetter CP, Schwarz EJ, Hess D, et al. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci USA. 2002; 99: 2199–204.

52. Abrams MB, Dominguez C, Pernold K, et al. Multipotent mesenchymal stromal cells attenuate chronic inflammation and injury-induced sensitivity to mechanical stimuli in experimental spinal cord injury. Restor Neurol Neurosci. 2007; 27: 307–21.

53. Pereira RF, O’Hara MD, Laptev AV, et al. Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. Proc Natl Acad Sci USA. 1998; 95: 1142–7.

54. Chopp M, Li Y, Zhang ZG. Direct evidence of mesenchymal stem cell transdifferentiation. Stem Cells Dev. 2009; 18: 129–37.

55. Krause U, Harter C, Seckinger A, et al. Intravenous delivery of autologous mesenchymal stem cells limits infarct size and improves left ventricular function in the infarcted porcine heart. Stem Cells Dev. 2007; 16: 31–7.

56. Tögel F, Westenfelder C. Stem cells in acute kidney injury repair. Minerva Urol Nefrol. 2009; 61: 205–13.

57. Lee RH, Seo MJ, Reger RL, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. Proc Natl Acad Sci USA. 2006; 103: 17438–43.

58. Ezquer F, Ezquer M, Simon V, et al. Endovenous administration of bone-marrow-derived multipotent mesenchymal stromal cells prevents renal failure in diabetic mice. Biol Blood Marrow Transplant. 2009; 15: 1354–65.

59. Gao J, Dennis JE, Muzic RF, et al. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs. 2001; 169: 12–20.

60. Schrepfer S, Deuse T, Reichenspurher H, et al. Stem cell transplantation: the lung barrier. Transplant Proc. 2007; 39: 573–6.

61. Kidd S, Spaeth E, Dembinski JL, et al. Direct evidence of mesenchymal stem cell tropism for tumor and Wounding microenvironments using in vivo bioluminescent imaging. Stem Cells. 2009; 27: 2614–23.

62. Lee RH, Pulin AA, Seo MJ, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009; 5: 54–63.

63. Lee JW, Fang X, Gupta N, et al. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. Proc Natl Acad Sci USA. 2009; 106: 16357–62.

64. Oh JY, Kim MK, Shin MS, et al. The anti-inflammatory and anti-angiogenic role of mesenchymal stem cells in corneal wound healing following chemical injury. Stem Cells. 2008; 26: 1047–55.

65. Timmers L, Lim SK, Arslan F, et al. Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem Cell Res. 2007; 1: 129–37.

66. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol. 1996; 166: 585–92.

67. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem. 2006; 98: 1076–84.

68. Ylöstalo J, Bazhanov N, Prockop DJ. Reversible commitment to differentiation by human multipotent stromal cells in single-cell-derived colonies. Exp Hematol. 2008; 36: 1390–402.

69. Gunw WG, Conley A, Deininger L, et al. A crosstalk between myeloma cells and marrow stromal cells stimulates production of DKK1 and interleukin-6: a potential role in the development of lytic bone disease and tumor progression in multiple myeloma. Stem Cells. 2006; 24: 986–91.

70. Ren G, Zhang L, Zhao X, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008; 2: 141–50.

71. Romieu-Mourez R, François M, Boivin MN, et al. Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol. 2009; 182: 693–73.

72. Wisniewski HG, Vilcek J. Cytokine-induced gene expression at the crossroads of innate immunity, inflammation and fertility: TSG-6 and PTX3/TSG-14. Cytokine Growth Factor Rev. 2004; 15: 129–46.

73. Mahoney DJ, Mikecz K, Ali T, et al. TSG-6 regulates bone remodeling through inhibition of osteoblastogenesis and osteoclast activation. J Biol Chem. 2008; 283: 25952–62.

74. Halkos ME, Zhao QZ, Kerendi F, et al. Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. Basic Res Cardiol. 2008; 103: 525–56.

75. Iso Y, Spees JL, Serrano C, et al. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. Biochem Biophys Res Commun. 2007; 354: 700–6.

76. Wolf D, Reinhard A, Seckinger A, et al. Regenerative capacity of intravenous autologous, allogeneic and human mesenchymal stem cells in the infarcted pig myocardium-complicated by myocardial tumor formation. Scand Cardiovasc J. 2009; 43: 39–45.

77. Mirotou M, Zhang Z, Deb A, et al. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. Proc Natl Acad Sci USA. 2007; 104: 1643–8.

78. Hung SC, Pochampally RR, Chen SC, et al. Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis. Stem Cells. 2007; 25: 2363–70.

79. Block GJ, Ohkouchi S, Fung F, et al. Multipotent stem cell cultures (MSCs) are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1 (STC-1). Stem Cells. 2009; 27: 670–8.

80. Wang Y, Huang L, Abdelrahim M, et al. Stanniocalcin-1 suppresses superoxide generation in macrophages through induction of mitochondrial UCPR. J Leukoc Biol. 2009; 86: 981–8.

81. Huang L, García G, Lou Y, et al. Anti-inflammatory and renal protective actions of stanniocalcin-1 in a model of anti-glomerular basement membrane glomerulonephritis. Am J Pathol. 2009; 174: 1368–78.

82. Le Blanc K, Rasmussen I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004; 363: 1439–41.

83. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and
disease. Nat Rev Immunol. 2008; 8: 726–36.

84. Bernardo ME, Locatelli F, Fibbe WE. Mesenchymal stromal cells. Ann NY Acad Sci. 2009; 1176: 101–17.

85. Ren G, Helwani FM, Verma S, et al. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. Stem Cells. 2009; 27: 1954–62.

86. Ratei M, Helwani FM, Verma S, et al. Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. J Immunol. 2009; 182: 5994–6002.

87. François M, Romieu-Mourez R, Stock-Martineau S, et al. Mesenchymal stromal cells cross-present soluble exogenous antigens as part of their antigen-presenting cell properties. Blood. 2009; 114: 2632–8.

88. English K, Ryan JM, Tobin L, et al. Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells. Clin Exp Immunol. 2009; 156: 149–60.

89. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007; 132: 2169–80.

90. Theuma P, Fonseca VA. Inflammation, insulin resistance, and atherosclerosis. Metab Syndr Relat Disord. 2004; 2: 105–13.

91. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res. 2003; 18: 696–704.

92. Crisan M, Yap S, Castellia L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008; 3: 301–13.

93. Diaz-Flores L, Gutiérrez R, Madrid JF, et al. Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol Histopathol. 2009; 24: 909–69.

94. Krueger M, Bechmann I. CNS pericytes: concepts, misconceptions, and a way out. Glia. 2010; 58: 1–10.

95. Spees JL, Olson SD, Whitney MJ, et al. Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci USA. 2006; 103: 1283–8.

96. Park IH, Daley GQ. Human iPS cell derivation/reprogramming. Curr Protoc Stem Cell Biol. 2009; 4: 1–8.

97. Kidd S, Spaeth E, Klop A, et al. The (in) auspicious role of mesenchymal stromal cells in cancer: be it friend or foe. Cytotherapy. 2008; 10: 657–67.

98. García S, Martín MC, de la Fuente R, et al. Pittfalls in spontaneous in vitro transformation of human mesenchymal stem cells. Exp Cell Res. 2010; 316: 1648–50.

99. Fang L, Gao XM, Moore XL, et al. Differences in inflammation, MMP activation and collagen damage account for gender difference in murine cardiac rupture following myocardial infarction. J Mol Cell Cardiol. 2007; 43: 535–44.

100. Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther. 2009; 17: 939–46.