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Human Embryonic Stem Cell Derived Mesenchymal Stromal Cells

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Abstract

Mesenchymal stromal cells (MSCs) originally isolated from bone marrow have multipotent differentiation potential, and favorable immunomodulatory and anti-inflammatory properties that make them very attractive for regenerative cellular therapy. Cells with similar phenotypic characteristics have now been derived from almost all fetal, neonatal and adult tissues; furthermore, more recently similar cells have also been generated from human embryonic stem cells (ESCs). Generation of MSCs from human ESCs provides an opportunity to study the developmental biology of human mesenchymal lineage generation *in vitro*. Generation of bone and cartilage from human ESC-derived MSCs and their functional characterization, both *in vitro* and *in vivo*, is also an active area of investigation as ESCs could provide an unlimited source of MSCs for potential repair of bone and cartilage defects. MSCs from adult sources are being investigated in numerous phase I-III clinical trials for a wide variety of indications, mainly based on their immunomodulatory properties. Our group and others have shown MSCs derived from human ESCs possess immunomodulatory properties similar to bone marrow-derived MSCs. Immunomodulatory properties of ESC-derived MSCs could prove to be highly valuable for their potential clinical applications in the future as derivatives of human ESCs have already entered clinical arena in the context of phase-I clinical trials.

Keywords

Embryonic stem cells; mesenchymal stromal cells; mesenchymal stem cells

Mesenchymal stromal cells

Mesenchymal stromal/stem cells (MSCs), originally isolated from bone marrow more than four decades ago, are multipotent cells capable of differentiating into cells of mesenchymal lineage such as bone, fat and cartilage^{1,2}. These cells are present at a very low frequency in unprocessed bone marrow and thus, there are no well-accepted criteria for their direct isolation and furthermore, their true identity *in vivo* is still not well known. Instead, what is essentially known as MSCs are a heterogeneous population of fibroblast like cells that are plastic adherent and are generated through *ex vivo* culture expansion. Among this population only a minority fulfills the strict criteria for stem cell definition, i.e. self-renewal and multi-lineage differentiation potential³. Although MSCs were originally isolated from bone marrow, populations of cells with similar phenotypic characteristics have also been isolated

from a wide variety of other adult tissues such as adipose tissue⁴, skeletal muscle⁵ and dental pulp⁶; from neonatal tissues such as placenta⁷ and umbilical cord blood⁸; and from fetal tissues such as liver, blood and bone marrow⁹. Due to uncertainties regarding how to define and/or characterize MSCs, a group of experts set forth the criteria for MSCs based on a combination of their culture properties, cell surface phenotypic markers and functional differentiation potential¹⁰. According to these widely accepted criteria, MSCs grow as plastic adherent cells, do not express hematopoietic markers such as CD34 and CD45, express a number of cell surface molecules including CD73, CD90 and CD105, and have a tri-lineage differentiation potential into fat, cartilage and bone *in vitro*. The differentiation potential of MSCs into other mesenchymal and non-mesenchymal cell types, a highly debated topic, has not been included as part of these criteria.

Tissue regenerative properties of mesenchymal stromal cells

MSCs have generated a lot of attention in the field of regenerative medicine not only due to their potential to differentiate into a variety of cell types but also because of their ability to migrate to sites of injury or inflammation after intravenous infusion and participate in tissue regeneration. For example, MSCs stimulate proliferation and differentiation of resident progenitor cells, and promote recovery of damaged tissues through secretion of a variety of cytokines and growth factors, and secretion of extracellular matrix components¹¹⁻¹⁴. Another intriguing property of *ex vivo* expanded MSCs is their ability to modulate the immune response *in vitro* and *in vivo* through interaction with a broad range of immune cells including T-lymphocytes, B-lymphocytes, natural killer and dendritic cells¹⁵⁻¹⁸. A variety of *in vitro* studies, have shown that MSCs possess the ability to suppress activation and proliferation of T-lymphocytes^{19,20}. MSCs are also assumed to be not targets of cytotoxic T cells or NK cells^{21,22} and thus, they could be potentially tolerated when transplanted over major histocompatibility complex barriers in humans²³. The mechanisms of action of MSCs in immunomodulation are through both direct cell-cell contact and through secretion of a variety of cytokines and paracrine factors. One of the more intriguing properties of *ex vivo* expanded MSCs is their ability to modulate the immune system and attenuate tissue damage caused by excessive inflammation²⁴; recent data strongly suggest that MSCs possess potent anti-inflammatory effects through interaction with the innate immune system cells such as macrophages^{25,26}. Again, many of these interactions are mediated through paracrine effects and secretion of anti-inflammatory cytokines²⁷⁻²⁹. Thus, in contrast to pharmacological drugs or biological therapeutics, such as monoclonal antibodies, MSCs exert their beneficial effects through many different mechanisms which make them potentially beneficial for a wide range of therapies.

Clinical experience with mesenchyme stromal cells

Autologous and allogeneic party bone marrow-derived MSCs have been investigated extensively in the context of hematopoietic stem cell transplantation either for enhancement of HSC engraftment^{30,31} or for prevention and/or treatment of graft versus host disease (GVHD). Indeed, the most compelling results have come from phase II studies in steroid-refractory acute GVHD³², and MSCs are closest to regulatory approval for use in acute GVHD pending the results of the now fully-accrued phase III trials²⁴. Interestingly, experience in using MSCs from third party donors have shown that use of MSCs from a donor different than that of the donor of hematopoietic stem cells (third party MSCs) is safe and as efficacious as MSCs from original donors or haplo-identical donors³². This has opened the way for use of third party MSCs from universal donors for treatment of other immune/inflammatory mediated disorders such as Crohn's disease³³. So far, culture-expanded MSCs derived from (autologous, allogeneic, or third party) bone marrow, and to a more limited extent, MSCs derived from other tissues such as fat have been used in

numerous phase I-II trials for a variety of non-hematological indications including treatment of patients with metachromatic leukodystrophy and Hurler's disease ³⁴, osteogenesis imperfecta ³⁵, myocardial infarction ^{36,37}, chronic obstructive pulmonary disease ³⁸, amyotrophic lateral sclerosis ³⁹, stroke ⁴⁰, refractory wounds ⁴¹, diabetes mellitus ⁴², systemic sclerosis ⁴³, and systemic lupus erythematosus ⁴⁴ among others. In all of these trials MSCs demonstrated an impressive safety record when used over a wide range of cell doses and administration frequencies ⁴⁵. Since many of these trials proved that MSCs from third-party donors could be used without any HLA-typing it makes it theoretically feasible that a few lines of clinical-grade human ESCs could be used as a renewable source for generation of sufficient MSCs for clinical applications for a large number of patients.

Derivation of mesenchymal stromal cells from human embryonic stem cells

Upon completion of my clinical and research fellowship at National Heart, Lung and Blood Institute I joined the Bone Marrow Transplant program at University of Wisconsin-Madison as a junior faculty. A major reason for this move to Madison, the birth place for human embryonic stem cell research⁴⁶, was the fact that I intended to establish a laboratory research program in this new field to specifically investigate human hematopoiesis using this highly relevant model system. This coincided with the first publication about the potential of third party bone marrow derived MSCs for treatment of refractory graft versus host disease ⁴⁷, a major complication after bone marrow transplantation. The fact that HLA-matching is not needed for MSC transplantation and thus a universal bone marrow donor can provide cells for a number of recipients sparked an interest in me to embark on laboratory experiments to derive mesenchymal stromal cells from human ESCs. The National Blood Foundation (NBF) award, coinciding with my K08 award, affirmed a sense of confidence in my ability to garner approval of my hypotheses and research plans from a group of colleague scientists. Indeed, NBF award was my first peer reviewed funding specifically directed towards my research on ESC-derived MSCs; now several years later my whole laboratory and translational research is focused on MSCs. As a bone marrow transplant physician with an interest in novel cellular therapies my goal was to develop a methodology for generation of MSCs from human ESCs that could facilitate its translation into a potential clinical product, considering the regulatory requirements by FDA for cellular therapy products. For example, although lack of animal-derived feeder cells is not a mandatory requirement in cell production it could greatly facilitate transition to the clinic. Thus, with the help of NBF seed money soon our group reported generation of cells from human ESCs that could be defined as MSCs according to the ISCT criteria for MSCs ¹⁰, without the use of any animal feeder cells ⁴⁸.

In general there are three methods for differentiation of ESCs. In the embryoid body (EB) method ESCs are grown into three-dimensional cellular aggregates which can then be differentiated into ectodermal, mesodermal and endodermal tissues based on differentiation medium and growth factors added. However, such differentiations are usually non-uniform and heterogeneous with multiple differentiated cell lineages present simultaneously. Another popular methodology is to use feeder cells to direct differentiation of ESCs towards specific lineages. For example, OP9 cells (a murine bone marrow stromal cell line), is commonly used for differentiation of ESCs towards mesodermal cells such as hematopoietic cells ⁴⁹. Another two-dimensional differentiation culture method is to use extracellular matrix (ECM, such as Matrigel, collagen or gelatin) in combination with specific cytokines and growth factors in culture medium.

A few years prior to studies in which generation of cells with *bona fide* characteristics of MSCs from ESCs were reported a few studies reported generation of mesenchymal-like cells that now in retrospect can be considered to be MSCs despite the fact that those cells

were not fully characterized or labeled as MSCs⁵⁰⁻⁵². For example, Stojkovic et al. showed that fibroblast-like cells that formed spontaneously in human ESC cultures, designated human ESC-derived fibroblasts, express MSC cell surface markers such as CD44 and CD90 but their differentiation potential into mesenchymal lineages was not further investigated⁵¹. However, Barberi et al. were the first to show mesenchymal-looking cells developed after 40 days of co-culturing human ESCs with OP9 cells, had characteristics typical of MSCs⁵³ based on expression of cell surface markers CD29, CD44, CD73, CD105, and their differentiating into bone, cartilage and fat. Later, Olivier et al. reported the Raclure method to generate MSCs from human ESCs, a method that did not need co-culturing with OP9, or any other supportive feeder cells⁵⁴. Lian et al. also reported a method to expand MSCs from the CD105+/CD24- population of cells differentiated from human ESCs⁵⁵, without a co-culture method. Although originally we used OP9 cells to derive MSCs from ESCs⁵⁶ later we developed a methodology to generate MSCs from human ESCs that did not include use of OP9 cells or any other animal feeder layer⁴⁸; such a modification removes a potential hurdle for their ultimate clinical application in the future. Not surprisingly, cells reported by different groups do not have the exact same phenotypic characteristics because even minor differences in the culture methodology can cause minor or major differences in the phenotype of generated cells. For example, cells generated by Olivier et al.⁵⁴ expressed typical markers of MSCs such as CD73 and CD105 but unlike our ESC-derived MSCs^{48,56}, those cells also expressed SSEA4⁴⁶. Presence of SSEA4 could pose a challenge as this marker is expressed on undifferentiated human ESCs and thus could confound the assessment of the purity of MSCs. Similarly, MSCs generated by Hwang et al.⁵⁷ exhibited many markers of MSCs including CD29 and CD105, but lacked CD73, a typical marker expressed by bone marrow derived MSCs¹⁰. This variability in cell surface marker expression could be due to many known and unknown factors. It will be very challenging to determine if, and how, such phenotypic differences in cell surface markers could impact the functional characteristics of MSCs. Aside from the potential clinical utility of human ESC-derived MSCs (discussed below) one of the obvious applications of this development is that it provides a new platform to study basic human developmental processes by allowing investigation of the development of mesodermal, or any other tissues, *in vitro*. Recapitulating development of tissues in culture dishes under our direct observation provides an unprecedented opportunity to study the earliest stages of human development *in vitro*⁴⁹.

Potential applications of ESC-derived MSCs

ESC-derived MSCs are of huge interest as they could be used for many non-clinical, and in the future, potentially clinical applications. For example, conventionally, human ESCs are derived and propagated on feeder cells, mainly murine embryonic fibroblasts (MEF)⁴⁶. Indeed, human ESC derived MSCs have now shown to be capable of functioning as autogenic or allogeneic feeder layers for derivation and/or expansion of human ESCs⁵⁸. Indeed, the ability to derive and use MSC feeder layers derived from human ESCs or from human adult bone marrow⁵⁹, instead of animal-derived feeder cells (i.e. MEF) is advantageous and potentially provides a safer source of cells for clinical applications.

In addition to studies on generation of MSCs from human ESCs, there has been a flurry of studies in which generation of derivative of MSCs, i.e. osteoblasts and chondrocytes, from human ESCs have been described⁶⁰⁻⁶⁵. However, it is not known if in these methodologies MSCs were only transiently present in the culture and thus went undetected or bone or cartilage lineages were generated without going through an MSC intermediate phase. In any case these studies provide unique insight into the osteogenesis and chondrogenesis *in vitro* and a potentially unlimited source for reconstructive surgery applications, including bone or cartilage repair⁶⁶. However, it can be also envisioned that ESC-derived MSCs could be

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directly implanted into bone or cartilage assuming the host tissue environment provides necessary signals towards their final differentiation into bone and cartilage⁶⁷. Although our laboratory was not the first to report the generation of MSCs from human ESCs our group was the first to report on the immunological characteristics of these cells⁴⁸. We showed that human ESC-derived MSCs possess immunological properties very similar to BM-derived MSCs, such as expression of HLA Class I antigens and lack of expression of HLA Class II antigens or co-stimulatory molecules, lack of capability to stimulate third party lymphocytes, and being able to suppress T-cell proliferation in mixed lymphocyte culture assays. Since our original report many other groups have expanded on our original observations and shown the immunosuppressive properties of ESC-derived MSCs both *in vitro*⁶⁸ and *in vivo*⁶⁹. Indeed the immunomodulatory and anti-inflammatory properties of MSCs is one of the most common reasons for their use in clinical trials. It is estimated that several thousand patients have so far have received BM-derived MSCs⁷⁰. MSCs derived from other sources such as adipose tissue, placenta, umbilical cord and even fetal tissues have also been used but to a much more limited extent⁴⁵. It can be argued that human ESCs could provide an unlimited source of MSCs for clinical application and thus obviate the need for invasive procedures to obtain MSCs from adult human volunteers. However, it should be noted that due to proliferative expansion of MSCs from adult sources usually small amounts of starting material is enough for treating potentially hundreds of patients. Also, tissues such as fat that will be otherwise discarded after surgery could be a source of MSCs. It should be also emphasized that although use of third party MSCs has been shown to be safe for specific indications such as GVHD, there will be certain clinical scenarios in which long-term engraftment of MSCs will be desirable, such as for repair of bone defects. In these situations use of autologous MSCs derived from adult tissues or MSCs derived from HLA-matched human ESC lines could prove to be advantageous. Nevertheless, ESC-derived MSCs remain as a potential therapeutic option for unique reasons. It can be envisioned that MSCs derived from a pluripotent stem cell line (either embryonic stem cell or induced pluripotent stem cells) could be used to enhance engraftment of other types of cells derived from those lines. Similar to utilization of BM-MSCs for enhancement of hematopoietic engraftment ESC-derived MSCs could be also used for potential enhancement of ESC-derived HSCs. MSCs are also being investigated as potential facilitators of solid organ transplantation^{71,72}. So, it could be also envisioned that MSCs derived from a pluripotent stem cell line could be used to enhance/promote engraftment of other cells, such as cardiomyocyte or pancreatic islet cells, derived from the same cell line.

However, before human ESC-derived MSCs reach clinic there are many issues that need to be resolved. For example, similar to the situation with adult tissue derived MSCs it is not clear if ESC-derived MSCs at different passages (passage-0 arbitrarily defined as the population that is homogenously looking like adult MSCs) possess different biological effects and what are the optimal passage of MSCs before they lose their biological properties. Furthermore, in the case of human ESCs as the source material for MSC derivation there will be an added layer of complexity as there will be ESC-inherent variables such as the passage number of human ESCs or the way they were derived that could have an effect on the biological properties of their derivatives (i.e. MSCs).

Safety Concerns

Infusion of *ex vivo* culture expanded MSCs from adult human bone marrow have been shown an impressive safety record of MSCs in all clinical trials thus far⁴⁵. Furthermore, transplantation of MSCs derived from other adult, neonatal and fetal tissues have been shown to be safe too due to the fact that these cells are considered to be not only non-immunogenic but actually elicit an immunomodulatory effect in the recipient. Based on the similar profile of immunomodulatory properties of human ESC-derived MSCs shown by our

group and others it can be assumed that if MSCs can be generated from a few human ESC lines under clinically acceptable conditions they could provide an alternative to adult tissue-derived MSCs and could be used as universal donor cells. However, use of any derivative of human ESCs carries some major concerns. For example, undifferentiated human ESCs, by definition, generate teratomas when transplanted into immunodeficient animals⁷³, a major concern for any clinical application of derivatives of human ESCs⁷⁴. However, derivation of MSCs from human ESCs generates highly pure population of cells in contrast to other types of cells⁷⁵⁻⁷⁷. One reason might be the fact that generation and expansion of MSCs from human ESCs, and any other sources, involves repeated passaging of cells in culture conditions that are not permissible for growth of human ESCs. This is in contrast to other derivatives of ESCs, such as cells of cardiac or neural lineages, in which repeated passaging is not part of their differentiation processes. In these circumstances persistence of undifferentiated ESCs could pose a major challenge to the safety of cellular therapy product.

Conclusion

Derivative of human ESCs provides the next frontier for novel cellular therapies. MSCs derived from human ESCs could provide an unlimited source of cells for potential clinical applications. These cells could be used based on their immunomodulatory properties, similar to bone marrow derived MSCs, or could be further differentiated into bone and cartilage before implantation. Furthermore, immunomodulatory and tissue protective effects of these cells provide a rationale for their co-transplantation with cells/tissues derived from the same pluripotent stem cell lines. Generation of MSCs from ESCs using clinically applicable methodologies could expand and facilitate use of derivatives of ESCs in the field of regenerative medicine.

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References

1. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 1968; 6:230–47.
2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284:143–7. [PubMed: 10102814]
3. Dominici M, Paolucci P, Conte P, Horwitz EM. Heterogeneity of multipotent mesenchymal stromal cells: from stromal cells to stem cells and vice versa. Transplantation. 2009; 87:S36–42. [PubMed: 19424002]
4. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001; 7:211–28. [PubMed: 11304456]
5. Williams JT, Southerland SS, Souza J, Calcutt AF, Cartledge RG. Cells isolated from adult human skeletal muscle capable of differentiating into multiple mesodermal phenotypes. Am Surg. 1999; 65:22–6. [PubMed: 9915526]
6. Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000; 97:13625–30. [PubMed: 11087820]
7. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells. 2004; 22:1338–45. [PubMed: 15579651]
8. Bieback K, Kern S, Kluter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells. 2004; 22:625–34. [PubMed: 15277708]

9. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood*. 2001; 98:2396–402. [PubMed: 11588036]
10. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8:315–7. [PubMed: 16923606]
11. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood*. 2003; 101:2999–3001. [PubMed: 12480709]
12. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol*. 2007; 213:341–7. [PubMed: 17620285]
13. Keating A. Mesenchymal stromal cells. *Curr Opin Hematol*. 2006; 13:419–25. [PubMed: 17053453]
14. Le Blanc K, Pittenger M. Mesenchymal stem cells: progress toward promise. *Cytotherapy*. 2005; 7:36–45. [PubMed: 16040382]
15. Stagg J, Galipeau J. Immune plasticity of bone marrow-derived mesenchymal stromal cells. *Handb Exp Pharmacol*. 2007; 45:66.
16. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood*. 2007
17. Le Blanc K, Ringden O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005; 11:321–34. [PubMed: 15846285]
18. Prockop DJ, Olson SD. Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions. *Blood*. 2007; 109:3147–51. [PubMed: 17170129]
19. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood*. 2002; 99:3838–43. [PubMed: 11986244]
20. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol*. 2003; 57:11–20. [PubMed: 12542793]
21. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol*. 2003; 31:890–6. [PubMed: 14550804]
22. Rasmusson I, Ringden O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. *Transplantation*. 2003; 76:1208–13. [PubMed: 14578755]
23. Le Blanc K, Gotherstrom C, Ringden O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Norden-Lindeberg S, Jansson M, Dalton A, Astrom E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation*. 2005; 79:1607–14. [PubMed: 15940052]
24. Battiwalla M, Hematti P. Mesenchymal stem cells in hematopoietic stem cell transplantation. *Cytotherapy*. 2009; 11:503–15. [PubMed: 19728189]
25. Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. *Exp Hematol*. 2009; 37:1445–53. [PubMed: 19772890]
26. Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med*. 2009; 15:42–9. [PubMed: 19098906]
27. Block GJ, Ohkouchi S, Fung F, Frenkel J, Gregory C, Pochampally R, DiMattia G, Sullivan DE, Prockop DJ. Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. *Stem Cells*. 2009; 27:670–81. [PubMed: 19267325]
28. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. 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. [PubMed: 19570514]
29. Oh JY, Roddy GW, Choi H, Lee RH, Ylostalo JH, Rosa RH Jr, Prockop DJ. Anti-inflammatory protein TSG-6 reduces inflammatory damage to the cornea following chemical and mechanical injury. *Proc Natl Acad Sci U S A*. 2010
 30. Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI. Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. *Bone Marrow Transplant*. 1995; 16:557–64. [PubMed: 8528172]
 31. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR Jr, Moseley AB, Bacigalupo A. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant*. 2005; 11:389–98. [PubMed: 15846293]
 32. Le Blanc K, Frasson F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet*. 2008; 371:1579–86. [PubMed: 18468541]
 33. Taupin P. OTI-010 Osiris Therapeutics/JCR Pharmaceuticals. *Curr Opin Investig Drugs*. 2006; 7:473–81.
 34. Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant*. 2002; 30:215–22. [PubMed: 12203137]
 35. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med*. 1999; 5:309–13. [PubMed: 10086387]
 36. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol*. 2004; 94:92–5. [PubMed: 15219514]
 37. Schuleri KH, Boyle AJ, Hare JM. Mesenchymal stem cells for cardiac regenerative therapy. *Handb Exp Pharmacol*. 2007; 195:218.
 38. Sueblinvong V, Weiss DJ. Cell therapy approaches for lung diseases: current status. *Curr Opin Pharmacol*. 2009; 9:268–73. [PubMed: 19349209]
 39. Deda H, Inci MC, Kurekci AE, Sav A, Kayihan K, Ozgun E, Ustunsoy GE, Kocabay S. Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell transplantation: a 1-year follow-up. *Cytotherapy*. 2009; 11:18–25. [PubMed: 19012065]
 40. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol*. 2005; 57:874–82. [PubMed: 15929052]
 41. Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, Takakura Y, Okuchi K, Nonomura A. Wound therapy by marrow mesenchymal cell transplantation. *Plast Reconstr Surg*. 2008; 121:860–77. [PubMed: 18317135]
 42. Abdi R, Fiorina P, Adra CN, Atkinson M, Sayegh MH. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes*. 2008; 57:1759–67. [PubMed: 18586907]
 43. Christopeit M, Schendel M, Foll J, Muller LP, Keysser G, Behre G. Marked improvement of severe progressive systemic sclerosis after transplantation of mesenchymal stem cells from an allogeneic haploidentical-related donor mediated by ligation of CD137L. *Leukemia*. 2008; 22:1062–4. [PubMed: 17972956]
 44. Liang J, Zhang H, Hua B, Wang H, Lu L, Shi S, Hou Y, Zeng X, Gilkeson GS, Sun L. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Ann Rheum Dis*. 2010; 69:1423–9. [PubMed: 20650877]

45. Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: An update on clinical trials with mesenchymal stem cells. *J Cell Physiol.* 2007; 211:27–35. [PubMed: 17226788]
46. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science.* 1998; 282:1145–7. [PubMed: 9804556]
47. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet.* 2004; 363:1439–41. [PubMed: 15121408]
48. Trivedi P, Hematti P. Derivation and immunological characterization of mesenchymal stromal cells from human embryonic stem cells. *Exp Hematol.* 2008; 36:350–9. [PubMed: 18179856]
49. Hematti P. Human embryonic stem cell-derived mesenchymal progenitors: an overview. *Methods in molecular biology.* 2011; 690:163–74. [PubMed: 21042992]
50. Xu C, Jiang J, Sottile V, McWhir J, Lebkowski J, Carpenter MK. Immortalized fibroblast-like cells derived from human embryonic stem cells support undifferentiated cell growth. *Stem Cells.* 2004; 22:972–80. [PubMed: 15536188]
51. Stojkovic P, Lako M, Stewart R, Przyborski S, Armstrong L, Evans J, Murdoch A, Strachan T, Stojkovic M. An autogeneic feeder cell system that efficiently supports growth of undifferentiated human embryonic stem cells. *Stem Cells.* 2005; 23:306–14. [PubMed: 15749925]
52. Wang Q, Fang ZF, Jin F, Lu Y, Gai H, Sheng HZ. Derivation and growing human embryonic stem cells on feeders derived from themselves. *Stem Cells.* 2005; 23:1221–7. [PubMed: 15955827]
53. Barberi T, Willis LM, Socci ND, Studer L. Derivation of multipotent mesenchymal precursors from human embryonic stem cells. *PLoS Med.* 2005; 2:e161. [PubMed: 15971941]
54. Olivier EN, Rybicki AC, Bouhassira EE. Differentiation of human embryonic stem cells into bipotent mesenchymal stem cells. *Stem Cells.* 2006; 24:1914–22. [PubMed: 16644919]
55. Lian Q, Lye E, Suan Yeo, K Khia, Tan E Way, Salto-Tellez M, Liu TM, Palanisamy N, El Oakley RM, Lee EH, Lim B, Lim SK. Derivation of clinically compliant MSCs from CD105+, CD24–differentiated human ESCs. *Stem Cells.* 2007; 25:425–36. [PubMed: 17053208]
56. Trivedi P, Hematti P. Simultaneous generation of CD34(+) primitive hematopoietic cells and CD73(+) mesenchymal stem cells from human embryonic stem cells cocultured with murine OP9 stromal cells. *Exp Hematol.* 2007; 35:146–54. [PubMed: 17198883]
57. Hwang NS, Varghese S, Zhang Z, Elisseeff J. Chondrogenic differentiation of human embryonic stem cell-derived cells in arginine-glycine-aspartate-modified hydrogels. *Tissue Eng.* 2006; 12:2695–706. [PubMed: 16995803]
58. Choo A, Ngo AS, Ding V, Oh S, Kiang LS. Autogeneic feeders for the culture of undifferentiated human embryonic stem cells in feeder and feeder-free conditions. *Methods Cell Biol.* 2008; 86:15–28. [PubMed: 18442642]
59. Cheng L, Hammond H, Ye Z, Zhan X, Dravid G. Human adult marrow cells support prolonged expansion of human embryonic stem cells in culture. *Stem Cells.* 2003; 21:131–42. [PubMed: 12634409]
60. Karp JM, Ferreira LS, Khademhosseini A, Kwon AH, Yeh J, Langer RS. Cultivation of human embryonic stem cells without the embryoid body step enhances osteogenesis in vitro. *Stem Cells.* 2006; 24:835–43. [PubMed: 16253980]
61. Cao T, Heng BC, Ye CP, Liu H, Toh WS, Robson P, Li P, Hong YH, Stanton LW. Osteogenic differentiation within intact human embryoid bodies result in a marked increase in osteocalcin secretion after 12 days of in vitro culture, and formation of morphologically distinct nodule-like structures. *Tissue Cell.* 2005; 37:325–34. [PubMed: 15979113]
62. Woll NL, Heaney JD, Bronson SK. Osteogenic nodule formation from single embryonic stem cell-derived progenitors. *Stem Cells Dev.* 2006; 15:865–79. [PubMed: 17253949]
63. Bielby RC, Boccaccini AR, Polak JM, Buttery LD. In vitro differentiation and in vivo mineralization of osteogenic cells derived from human embryonic stem cells. *Tissue Eng.* 2004; 10:1518–25. [PubMed: 15588411]

64. Toh WS, Yang Z, Liu H, Heng BC, Lee EH, Cao T. Effects of culture conditions and bone morphogenetic protein 2 on extent of chondrogenesis from human embryonic stem cells. *Stem Cells*. 2007; 25:950–60. [PubMed: 17218402]
65. Sottile V, Thomson A, McWhir J. In vitro osteogenic differentiation of human ES cells. *Cloning Stem Cells*. 2003; 5:149–55. [PubMed: 12930627]
66. zur Nieden NI. Embryonic stem cells for osteo-degenerative diseases. *Methods in molecular biology*. 2011; 690:1–30. [PubMed: 21042982]
67. Alsberg E, von Recum HA, Mahoney MJ. Environmental cues to guide stem cell fate decision for tissue engineering applications. *Expert Opin Biol Ther*. 2006; 6:847–66. [PubMed: 16918253]
68. Yen BL, Chang CJ, Liu KJ, Chen YC, Hu HI, Bai CH, Yen ML. Brief report--human embryonic stem cell-derived mesenchymal progenitors possess strong immunosuppressive effects toward natural killer cells as well as T lymphocytes. *Stem Cells*. 2009; 27:451–6. [PubMed: 18988708]
69. Sanchez L, Gutierrez-Aranda I, Ligerio G, Rubio R, Munoz-Lopez M, Garcia-Perez JL, Ramos V, Real PJ, Bueno C, Rodriguez R, Delgado M, Menendez P. Enrichment of Human ESC-Derived Multipotent Mesenchymal Stem Cells with Immunosuppressive and Anti-Inflammatory Properties Capable to Protect Against Experimental Inflammatory Bowel Disease. *Stem Cells*. 2010
70. Tolar J, Le Blanc K, Keating A, Blazar BR. Concise review: hitting the right spot with mesenchymal stromal cells. *Stem Cells*. 2010; 28:1446–55. [PubMed: 20597105]
71. Hematti P. Role of mesenchymal stromal cells in solid organ transplantation. *Transplant Rev (Orlando)*. 2008; 22:262–73. [PubMed: 18656340]
72. English K, French A, Wood KJ. Mesenchymal stromal cells: facilitators of successful transplantation? *Cell stem cell*. 2010; 7:431–42. [PubMed: 20887949]
73. Thomson JA, Odorico JS. Human embryonic stem cell and embryonic germ cell lines. *Trends Biotechnol*. 2000; 18:53–7. [PubMed: 10652509]
74. Baker M. FDA to vet embryonic stem cells' safety. *Nature*. 2008; 452:670. [PubMed: 18401364]
75. Dihne M, Bernreuther C, Hagel C, Wesche KO, Schachner M. Embryonic stem cell-derived neuronally committed precursor cells with reduced teratoma formation after transplantation into the lesioned adult mouse brain. *Stem Cells*. 2006; 24:1458–66. [PubMed: 16456136]
76. Cai J, Yi FF, Yang XC, Lin GS, Jiang H, Wang T, Xia Z. Transplantation of embryonic stem cell-derived cardiomyocytes improves cardiac function in infarcted rat hearts. *Cytherapy*. 2007; 9:283–91. [PubMed: 17464760]
77. Arnhold S, Klein H, Semkova I, Addicks K, Schraermeyer U. Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. *Invest Ophthalmol Vis Sci*. 2004; 45:4251–5. [PubMed: 15557428]