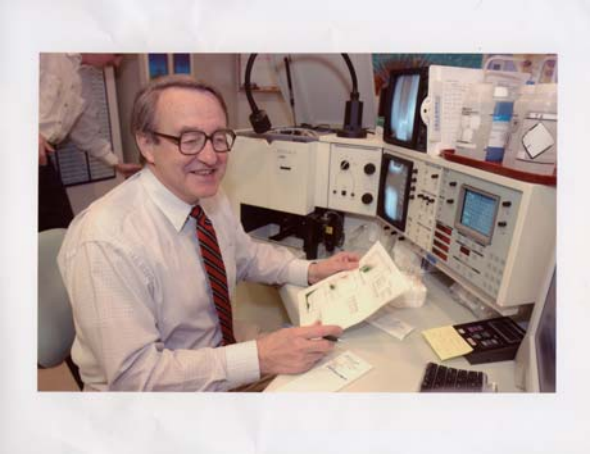

Darwin J. Prockop, M.D., Ph.D.
Professor and Director, Center for Gene Therapy

The Reparative Power of Multipotent Stromal Cells from Bone Marrow.

Darwin J. Prockop, Center for Gene Therapy, Tulane University
Health Sciences Center, New Orleans, LA 70112

Recent publications have demonstrated that most tissues contain stem-like progenitor cells that play a key role in the repair of tissue injury. When the endogenous stem/progenitor cells in a tissue are exhausted, they are supplemented by similar stem/progenitor cells from the bone marrow. A major focus has been on the stem/progenitor cells from bone marrow referred to as mesenchymal stem cells or multipotent stromal cells (MSCs). MSCs and similar cells from other tissues have been shown to repair tissues by differentiating so as to replace injured cells, by producing chemokines, and in part by cell fusion. However, there has been no obvious explanation for repeated observations that MSCs enhance repair of tissues in experimental models in which their level of engraftment is extremely low. We have recently found that MSCs can repair injured cells and tissues by two additional mechanisms: Stimulation of the proliferation and differentiation of stem cells that are endogenous to a tissue and by transfer of mitochondria or mitochondrial DNA to cells with non-functional mitochondria. Human MSCs infused into the hippocampus of immunodeficient mice stimulated proliferation of and neurogenesis by endogenous neural stem cells (Munoz et al. PNAS, 2005). Co-culture of human MSCs with a line of pulmonary epithelial cells with non-functional mitochondria generated clones of the epithelial cells with functional mitochondria as a result of active transfer of either mitochondria or mitochondrial DNA from the MSCs (Spees, Olson, et al., PNAS, 2006). More recently we observed (Lee et al., PNAS in press) that intravenously infused human MSCs lowered the blood sugar, increased mouse insulin and decreased morphological changes in the renal glomeruli of streptozocin-treated diabetic mice (NOD/SCID). The human MSCs engrafted into the pancreas and increased both the number of islets and the immunoreactive mouse insulin per islet. The human MSCs also engrafted into the kidney but it was not apparent whether the decrease in renal pathology was explained by direct action of the cells or by the decrease in blood sugar. Therefore there are now multiple strategies for developing new therapies for a broad range of diseases by enhancing one or more of the multiple mechanisms whereby MSCs normally repair tissues. Supported in part by grants from NIH grants AR48323, HL 073755, HL075161, and HL073252; HCA the Healthcare Company, and the Louisiana Gene Therapy Research Consortium.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Darwin J. Prockop, M.D., Ph.D.	Professor and Director, Center for Gene Therapy
eRA COMMONS USER NAME DPROCKOP	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Haverford College, Haverford, PA	A.B.	1951	Philosophy (Pre-Med)
Brasenose College, Oxford University	M.A.	1953	Animal Physiology
University of Pennsylvania, School of Medicine	M.D.	1956	Medicine
George Washington University	Ph.D.	1961	Biochemistry

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

1956-1957 Intern in Medicine, NY Hospital-Cornell Medical Center, New York, NY
1956-1961 Postdoctoral Fellow, Senior Investigator, National Heart Institute, National Inst. of Health, Bethesda, MD
1961-1972 Professor of Medicine, Biochemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, PA
1972-1986 Professor & Chair, Dept. Biochemistry, Univ. Medicine and Dentistry-Rutgers Medical School, Piscataway, NJ
1986-1996 Professor and Chairman, Dept. of Biochemistry & Molecular Biology; Director, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Philadelphia, PA
1996-2000 Professor and Director, Center for Gene Therapy, Allegheny University of the Health Sciences (now MCP Hahnemann University), Philadelphia, PA
2000- Professor of Biochemistry and Director, Center for Gene Therapy, Tulane University Health Sciences Center, New Orleans, LA

Academician of the Academy of Finland, 1990
Senior Humboldt Research Award, German Research Council, 1990-1995
Member, National Academy of Sciences, 1991
Member, National Institute of Medicine, 1992
Howley Prize for Research, Arthritis Foundation, 1992
Distinguished Alumnus: George Washington University, 1993; School of Medicine, University of Pennsylvania, 1994
Honorary Doctorates: University of Oulu, Finland, 1983; University of South Florida, 1993
Hopkins Medal, British Biochemical Society, 1998
Honorary Companion, University of Manchester, UK, 1999

B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

(from total of over 500):

1. D.J. Prockop. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276:71-74 (1997).

2. JR.F. Pereira, M.D. O'Hara, A.V. Laptev, K.W. Halford, M.D. Pollard, R. Class, D. Simon, K. Livezey and D.J. Prockop. Marrow stromal cells as a source of progenitor cells for non-hematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. Proc. Natl. Acad. Sci. 95:1142-1147 (1998).
3. S.A. Azizi, D. Stokes, B.J. Augelli, C. DiGirolamo and D.J. Prockop. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats. Similarities to astrocyte grafts. Proc. Natl. Acad. Sci. 95:3908-3913 (1998).
4. D.J. Prockop. The genetic trail to osteoporosis. N. Eng. J. Med. 338:1061-1062 (1998).
5. G.C. Kopen, D.J. Prockop and D.G. Phinney. Marrow stromal cells migrate throughout forebrain and cerebellum and they differentiate into astrocytes following injection into neonatal mouse brains. Proc. Natl. Acad. Sci. 96:10711-10716 (1999).
6. E.M. Horowtiz, D.J. Prockop, P.L. Gordon, W.W.K. Koo, L.A. Fitzpatrick, M.D. Neel, M.B. McCarville, P.J. Orchard, R.E. Pyeritz and M.K. Brenner. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. Blood 97:1227-1231 (2001).
7. E.H. Javazon, D.C. Colter, E.J. Schwarz and D.J. Prockop. Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single-cell-derived colonies than human marrow stromal cells. Stem Cells 19:219-225 (2001).
8. D.C. Colter, I. Sekiya and D.J. Prockop. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proceedings National Academy of Sciences 98:7841-7845 (2001).
9. C.P. Hofstetter, E.J. Schwarz, D. Hess, J. Widenfalk, D.J. Prockop and L. Olson. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. PNAS 99:2199-2204 (2002).
10. I. Sekiya, J.T. Vuoristo, B.L. Larson and D.J. Prockop. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. PNAS 99(7):4397-4402 (2002).
11. D. J. Prockop. Adult stem cells gradually come of age. Nature Biotechnology 20: 7-8 (2003).
12. J. L. Spees, S. D. Olson, J. Ylostalo, P. J. Lynch, J. Smith, A. Perry, A. Peister, M.-Y. Wang and D. J. Prockop. Differentiation, cell fusion and nuclear fusion ex vivo repair of epithelium by human adult stem cells from bone marrow stroma (hMSCs). Proceedings of the National Academy of Sciences, USA 100(5): 2397-2402 (2003)
13. D. J. Prockop. Further proof of the plasticity of adult stem cells and their role in tissue repair. Journal of Cell Biology, 160(6): 807-809 (2003).
14. C. A. Gregory, H. Singh, A. S. Perry and D. J. Prockop. The Wnt signaling inhibitor Dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow. The Journal of Biological Chemistry 278(30): 28067-28078 (2003).
15. D. J. Prockop, C. A. Gregory and J. L. Spees. One strategy for cell and gene therapy: Harnessing the power of adult stem cells to repair tissues. Proceedings of the National Academy of Sciences 100 (1): 11917-11923 (2003).
16. A. Peister, J. A. Mellad, B. L. Larson, B. M. Hall, L. F. Gibson and D. J. Prockop. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood. Oct. 30 (2003) Reprint 485
17. R.R. Pochampally, J.R. Smith, J. Ylostalo and D.J. Prockop. Serum deprivation of human marrow stromal cells (hMSCs) selects for a sub-population of early progenitor cells with enhanced expression of Oct-4 and other embryonic genes. Blood **103 (5):** 1647 – 1652 (2004).
18. G. Wang, B. A. Bunnell, R.G. Painter, B.C. Quinones, S.Tom, N. A. Lanson, Jr., J.L. Spees, D. Bertucci, A.Peister, D. J. Weiss, V.G. Valentine and D.J. Prockop. Adult Stem Cells from Bone Marrow Stroma Differentiate Into Airway Epithelial Cells: Potential Therapy for Cystic Fibrosis. Proceedings of the National Academy of Sciences **102:** 186-191 (2005)
19. R.R. Pochampally, B.T. Neville, E.J. Schwarz, M.M. Li and D.J. Prockop. Rat Adult Stem Cells (Marrow Stromal Cells) Engraft and Differentiate in chick Embryos without Evidence of Cell Fusion. Proceedings of the National Academy of Sciences **101(25):** 9282-9285 (2004).

20. C. A. Gregory, A. S. Perry, E. Reyes, A. Conley, W. G. Gunn and D. J. Prockop. Dkk-1 derived Synthetic Peptides and Lithium Chloride for the Control and Recovery of Adult Stem Cells from Bone Marrow. The Journal of Biological Chemistry 280: 2309-2323 (2005)

21. R.H. Lee, S. C. Hsu, J. Munoz, J.S. Jung, N.R. Lee, R. Pochampally and D. J. Prockop. A Subset of Human Rapidly-Self Renewing Marrow Cells (MSCs) Preferentially Engraft in Mice. Blood First Edition paper, pre-published online November 8, 2005; DOI 10.1182/Blood-2005-07-2701.

22. W. G. Gunn, A. Conley, P. L. Deininger, S. D. Olson, D. J. Prockop, and C. A. Gregory A Crosstalk Between Myeloma Cells and Marrow Stromal Cells Stimulates Production of DKK1 and IL-6: A Potential Role in the Development of Lytic Bone Disease and Tumor Progression in Multiple Myeloma. Stem Cells. November 17 [epub. Ahead of print] (2005).

23. J. R. Munoz, B. R. Stoutenger, A. P. Robinson, J. L. Spees and D. J. Prockop. Human Stem/Progenitor Cells from Bone Marrow Promote Neurogenesis of Endogenous Stem Cells in the Hippocampus of Mice. PNAS 102: 18171-18176 (2005).

C. Research Support

ONGOING PROJECTS:

(1) 1R01AR48323 (Darwin J. Prockop, M.D., Ph.D., P.I.) 19/21/01—7/31/06
NIH/NIAMS
Osteoprogenitors for Potential Therapy of OI.

Specific Aims (1) Use a series of antibodies to surface epitopes we have recently identified to prepare clonal and homogeneous preparations of RS cells from cultures of human MSCs. In the process, test the hypothesis that RS cells can be further fractionated to obtain homogeneous preparations of stem cells that are even more effective as osteoprogenitors for engraftment into bone. (2) Define the osteogenic potential *in vitro* of the RS cell preparations by assays of the rates of mineralization and assays of expressed genes by mRNA microarrays and proteomics. (3) Determine the osteogenic potential *in vivo* of the RS cell preparations by assays of differentiation into bone after subcutaneous implantation in vehicles or after systemic infusion into immunodeficient mice. (4) Determine the feasibility of correcting the gene defect in a patient's own RS cells, (a) by overexpression of a cDNA for the wildtype COL1A1 gene, or (b) by replacing a mutated COL1A1 gene by homologous recombination.

(2) R000 (Darwin J. Prockop, M.D. Ph.D., Project Leader) 1/01/02-12/31/07
(Board of Regents of LA)
HEF Center for Gene Therapy for Acquired and Genetic Diseases. Project 1: Adult Stem Cells for Therapy in Primates.

Specific Aims 1. Isolate and expand primate MSCs with the primate MSCs with the improved protocol our laboratory has recently developed to isolate and expand cultures of human MSCs that are high enriched for the earliest progenitor cells (RS cells). 2. Compare the primate MSCs in culture with human MSCs in their ability to expand rapidly and to differentiate into osteoblasts, chondrocytes, adipocytes, and neural cells. 3. Compare the primate MSCs to human MSCs in vivo in their ability to engraft into multiple tissues after systemic or intracranial infusion into immunodeficient mice. 4. When a recently purchased microPET instrument becomes operational, assay the engraftment of the primate cells first into immunodeficient mice and then into the same primates from which the cells were derived using real-time imaging with the microPET.

(3) 1 R01HL073755 (Darwin J. Prockop, M.D.,,Ph.D., P.I.) 7/01/03-6/30/07
NIH/NHLBI
Adult Stem Cells for Repair of Cardiac Damage.

SPECIFIC AIMS: The Specific Aims are: (1) Further characterize the special subclass of stem-like cells that we have recently isolated from cultures of MSCs and that have unusually long telomeres and propagate more rapidly than parallel samples of MSCs. We will test the hypothesis that the cells are pre-RS cells, *i.e*. precursors of the rapidly self-renewing cells (RS) cells that we previously identified as a sub-population of early progenitors in standard cultures of MSCs. (2) Use an *ex vivo* co-culture system to compare the ability of the putative pre-RS

cells, RS cells, mMSCs, and marrow mononuclear cells to repair hypoxic damage to cardiac cells either through direct differentiation of the cells or through cell fusion. (3) Isolate and characterize putative pre-RS cells, RS cells, mMSCs and mononuclear cells from rat bone marrow for testing in *in vivo* models of cardiac ischemia in Specific Aim 4. (4) Determine the ability of rMSCs, mMSCs, pre-RS, RS and unfractionated mononuclear cells from rat marrow to engraft into and produce functional improvement and reduce damage in rat models of acute myocardial infarction (ischemia-reperfusion) and coronary arteriogenesis (intermittent repetitive ischemia). Note: There is some development with the present application.

(4) 1 840 RR017447 6/1/03-5/31/08
(Darwin J. Prockop, M.D., Ph.D. (P.I.)
Preparation and Distribution of Adult Stem Cells

SPECIFIC AIMS: The aims are to establish a Center that will: (1) Prepare a continuous supply of human MSCs that are thoroughly quality tested and distribute them on request to other investigators at multiple institutions for research on the cells. (2) Prepare a similar continuous supply of rat MSCs for distribution to investigators at multiple institutions. (3) Prepare MSCs from human bone marrow aspirates sent to us by investigators at other institutions and return the quality-tested MSCs to the same investigators. Also, carry out quality testing of MSCs prepared by investigators at other institutions. (4) Develop improved methods for isolating and characterizing human, rat and mouse MSCs.

(5) 1 R01 HL073252 7/1/03 – 6/30/07

(Darwin J. Prockop, M.D., Ph.D., P.I.)

Culture and Lung Engraftment of Mesenchymal Stem Cells

Specific Aims are: (1) Elucidate the molecular events that permit and enhance *ex vivo* expansion of hMSCS. (2) Develop an *ex vivo* assay for the potential of hMSCs to repair injured lung by co-culturing hMSCs with lung cells that have been damaged by heat-chock and hypoxia. (3) Develop an *in vivo* assay for engraftment and differentiation of hMSCs to sites of tissue injury in lung. (4) Identify the properties of hMSCS that enhance their engraftment to specific sites of tissue injury in lung. Note: There is some overlap between this grant and the present application.

Completed Research Support

Funding Period

(1) 1R21-AR47161 (Darwin J. Prockop, M.D., P.I.) 7/1/00 – 6/30/02
NIH/NIAMS
Expansion of Stem Cells for Skeletal Diseases.

Specific Aims: (1) Determine whether the highly replicative cells we have obtained after 10-fold expansion of hMSCs retain their multipotentiality to differentiate into osteoblasts, chondrocytes, and adipocytes. (2) Isolate and characterize the secreted factors that require replication of hMSCs in culture. (3) Isolate the small, granular, and highly replicative cells we have identified in culture of hMSCs and define their surface epitopes.