REGENERATIVE STEM CELL THERAPY IN RETINAL DEGENERATION

Irfan Ahmed Siddiqui[1]and Dr. K. Babu Rao[2]

[1]Research Scholar, CMJ University, Shillong, Meghalaya

Principal Donbasco College Pharmacy, Guntur District, AP

Abstract:

Stem cell-based therapy has been tested for several diseases, including neurodegenerative disorders, such as Parkinson's disease, spinal cord injury, and multiple sclerosis in animal models. The replacement of lost neurons that are not physiologically replaced is pivotal for therapeutic success. In the eye, degeneration of neural cells in the retina are hallmarks of such wide-spread ocular diseases as AMD and RP. In these cases the primary cause of blindness is due to loss of photoreceptors. This can result from dysfunction in either the PRC or the underlying RPE that supports their survival.

Transplantation of RSC with the potential to generate new retinal cells provides an alternative approach to enable the replacement of lost PRC or RPE. Retinal stem cells may restore vision in patients who have degenerative retinal diseases by two possible means: 1) repopulation of the damaged retina (e.g., PRC); and/or 2) rescue of retinal neurons from further degeneration.[80] Different research groups have successfully isolated murine putative RSC from the ciliary margin (CM) and human RSC in the pars plana and pars plicata.[81,82] However, the transplantation of these cells in normal and degenerative rodent retina was only minimally successful due to the limited ability of the cells to invade and integrate into the host retina.[27] On the other hand, transplantation of immature post-mitotic rod precursors from developing retina (postnatal day 1) improves retinal integration.[83] The optimal result occurs when selected cells were biochemically committed but not yet morphologically differentiated. The capability of subretinally or intravitreously injected RSC to invade and integrate into the neural retina remains restricted to sites of retinal injury. Breakdown of physical barriers, such as the outer limiting membrane, and/or release of unknown neurotrophic factors, are most likely required to stimulate RSC integration.[84] To date only sparse data are available regarding factors that might stimulate migration, integration, and differentiation of RSC into the neural retina.

Keywords: *Retinal degeneration, neuro degenerative disorders, regenerative stem cells (RSCs), bone marrow stem cells (BMS), Retinal pigment epithelium (RPE), Transforming growth factor (TGF), Fibroblast growth factor (FGF).*

INTRODUCTION

It is assumed that neurotrophic factors, such as transforming growth factor (TGF-beta 3),[85] fibroblast growth factor (FGF),[86] or epidermal growth factor (EGF),[87,88] might play a role. Recent evidence has suggested that hepatocyte growth factor/scatter factor (HGF/SF), a pleiotrophic factor with mitogenic, and morphogenic activities, may also be involved in the development and maintenance of neurons and PRC.

The replacement of diseased RPE in AMD would be pivotal to protect or rescue the adjacent PRC. Unfortunately, no convincing animal model for AMD exists to date. Therefore, the sodium iodate ($NaIO_3$) model of RPE damage, established by G.E. Korte in 1984,[90] has been used to study at least the repopulation of bare areas of normal Bruch's membrane.[91] Briefly, the selective and patchy degeneration of the RPE monolayer after i.v. $NaIO_3$ injection is directly correlated to decreased visual function, decreased electrophysiological function and anatomical cell loss in the RPE .

The extent of the RPE damage is time and concentration-dependent. Interestingly, $NaIO_3$-damaged RPE cells express higher amounts of cytokine/growth factors involved in SC homing. After treatment with $NaIO_3$, murine RPE cells express higher levels of SDF-1, as well as other signaling factors (complement factor C3 and HGF/SF). SDF-1 is a chemokine whose receptor CXCR4 is expressed on bone marrow-derived progenitor cells and stem cells.[93] While there was no evident change in vascular endothelial growth factor and Rantes, there was increased expression of the cytokine leukocyte inhibitory factor, known to promote self-renewal in ESC.[94] Furthermore, supernatants of $NaIO_3$–damaged RPE exert a priming effect on BMSC migration *in vitro* as they enhanced their transwell migration.[94] These results provide evidence that damage to the RPE leads to production of soluble factors that can cause specific chemotaxis of BMSC and raise the possibility of their recruitment to the site of damage. These data support the possibility of using BMSC to replace damaged cells, especially RPE, in eyes with retinal degenerations. To investigate this further, we have undertaken endogenous as well as exogenous approaches using BMSC using the above described $NaIO_3$ model. Endogenous refers to existing bone marrow cells in the host while exogenous refers to adoptively transferred cells.

Sodium iodate model of retinal pligment epithelium (RPE) degeneration A-F, Autofluorescence in flat-mount whole-eye preparations of control (D) and sodium iodate-treated mice (A-C,E, and F). The top row (A-C) compared different doses of sodium iodate at 7 days postinjection (P1): 35 mg/kg (A), 50 mg/kg(B), and 70 mg/kg (C) of body weight, E, B, and F compare different times PI at the same dose (50 mg/kg) : 3 days PI (E): 7 days PI (B)L and 21 days PI (F). Beinning on 3 days PI, a patchy loss of RPE can be detected by the decrease in autofluorescence (black areas). The total area bare of RPE (autofluorescent areas) is dose dependent and increased over time (original magnification x 1000).

Review of literature

The use of stem cells to replace degenerated RPE cells has not yet demonstrated the ability to rescue photoreceptors cells at risk of damage. If stem cell differentiation and reconstitution of the damaged RPE monolayer occurs after photoreceptor degeneration, a rescue effect will not be possible. Alternatively, if the mobilization of endogenous stem cells occurs continuously or over a prolonged period of time, photoreceptor damage and/or rescue may be possible.[96]

The regenerative capability of BMSC in the ocular system is not only restricted to RPE replacement. Chiou et al. showed that BMSC have multilineage differentiation potential *in vitro* and differentiate into retinal cells and photoreceptor lineages after co-culture with RPE cells.[97] Other groups have followed different approaches to replace diseased RPE cells. Haruta and colleagues harvested RPE-like ESC *in vitro* and achieved functional improvement after subretinal transplantation into RCS rats.[98]

Only a small percentage of total bone marrow cells are chemoattracted to supernatants from damaged RPE *in vitro*, as well as into damaged RPE *in vivo*, the properties of this subset of BM-derived cells need to be considered. Recent data indicate that the $CD45^+$ population of stem cells is committed to hematopoietic lineages, while the $CD45^-$ population is believed to remain pluripotent and thus capable of differentiation into various non-hematopoietic tissues.

Kucia et al. showed that $CD45^-$ BMSC are comprised of subsets of cells already committed to skeletal muscle, heart muscle, liver and neural tissues.[55] These so called TCSC, more recently re-named very small embryonic-like cells (VSEL)[100] express Oct-4, a stem cell marker, in addition to markers of tissue-specific progenitors. These TCSC are mobilized into PB during organ injury.[101] SDF-1-based chemotactic isolation combined with RT-PCR analysis of mRNA revealed that early TCSC: 1) reside in the normal human and murine BM; 2) express CXCR4 on their surface; and 3) can be highly enriched in humans and mice after chemotaxis to an SDF-1 gradient. These studies were performed on freshly isolated cells, ruling out the potential contribution of culture-related transdifferentiated HSC or mesenchymal cells. In our experiments we found that Sca-1^+ $CD45^-$ BMSC are highly enriched in mRNA for retinal/RPE progenitors (Six-3, OTX, Pax-6, MITF; data not shown) and furthermore, that this is the subset of BMSC that has migrated in response to supernatants from damaged RPE in transwell assays. Thus, it appears that RPE-committed VSEL cells (approximately 0.05% of the population) are present within the Sca-1^+ $CD45^-$ subset of BMSC. This is supported by data from *in vitro* experiments using a co-culture of BMSC and RPE cells to trigger SC differentiation into the RPE-lineage .

Material and method

Two types of approaches can be used to promote stem-cell-mediated regenerative repair of RPE: endogenous and exogenous. Endogenously, RPE injury combined with pharmacologically enhanced growth factor-mediated mobilization lead to migration of BM-derived cells into the subretinal space. BMSC (c-kit^+), macrophages (F4/80) and leukocytes such as granulocytes, monocytes (CD11b) could be identified. Thereby, the number of c-kit^+ BMSC in the eye after $NaIO_3$ injection and mobilization increased dramatically compared to the mobilized control mice who did not have RPE damage.[91] The migrated BMSC had incorporated in a monolayer along the RPE four weeks after transplantation and expressed the RPE markers RPE-65 and MITF (Figure 2). These findings suggest that bone marrow-

derived stem cells are attracted to damaged RPE and are induced to differentiate into components of RPE. Mobilization enhances the outcome.

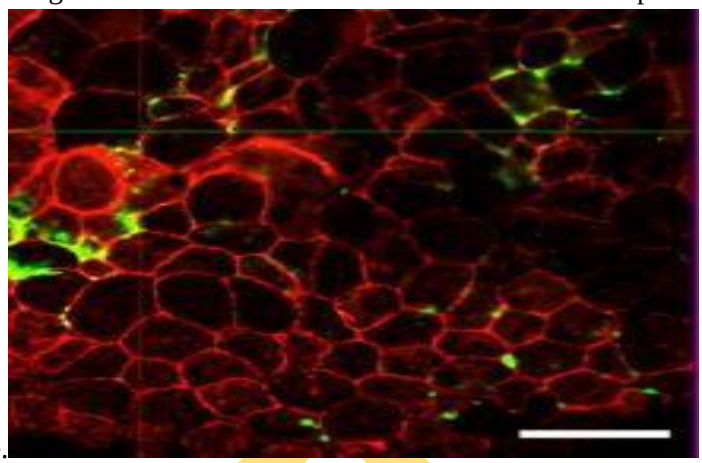

Figure 2 R??EPE-65 and MITF

Expression of RPE markers RPE-65 and MITF

The results above demonstrated that a physiological process is in place *in vivo* to recruit stem cells to the damaged RPE and that endogenous BM-derived cells are able to integrate into the damaged RPE and express markers of RPE differentiation. Nevertheless, the significant experimental damage to the RPE could not be repaired by this endogenous approach, nor does this endogenous program appear capable of repairing or preventing the progressive damage to the RPE that occurs in AMD and retinitis pigmentosa. Thus, it appears that such recruitment of endogenous cells may not be sufficient to physiologically repair significant damage to the RPE in the same fashion that recruitment of endogenous SC cannot repair major damage to spinal cord or heart.

To optimize number and availability of circulating BMSC, we then examined an exogenous approach for regeneration of damaged RPE. Additionally, this allows us to define the precise cell types involved using cell sorting as opposed to the mixture of stem cells and other BM-derived cells mobilized into the periphery with the endogenous approach. We injected FACS-sorted BMSC with the phenotype lin^- (negative for all lineages of differentiated BM cells), stem cell antigen 1 (Sca-1)-positive intravenously (i.v.) into $NaIO_3$ treated animals. BMSC could be detected in the subretinal space on Bruch's membrane in areas of RPE loss on day four after cell injection, whereas controls without $NaIO_3$ injection showed no BMSC. The double staining for Sca-1 and green fluorescence protein (GFP) confirms the BM origin of the cells systemically transferred and confirms that HSC home to the area of damaged RPE after $NaIO_3$ injection. One and two weeks after transfer, BMSC could be identified in the subretinal space but they did not express RPE markers. Immunocytochemical staining showed the expression of RPE-65 in BMSC four and six weeks after transplantation. These results suggest that, as with the endogenous cells, BMSC injected systemically into the host home to the site of damage where they integrate and express markers of RPE differentiation in a time-dependent fashion.[95].

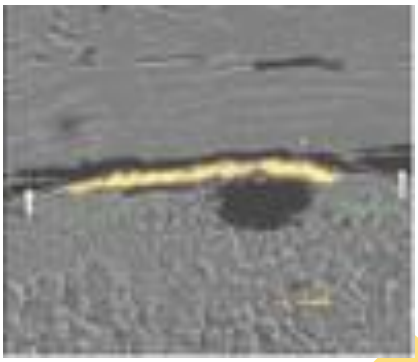

Figure 3 Immunocytochemical staining of vertical sections of a GFP chimeric

Mouse eye four weeks after $NaIO_3$ treatment and BMC mobilization

A third route for BMSC delivery is by direct subretinal injection. It is observed that subretinally injected BMSC integrated into the RPE and expressed markers of differentiation (e.g., RPE65). The optimal route for SC delivery remains to be determined. Concentrating the cells might provide a kinetic advantage for incorporation of the cells into the altered tissue. Thereby, the cells would not have to home to sites of damage from the circulation.

BMSC changed their morphology from round to epithelial–like and expressed the epithelial markers cytokeratin, MITF - expressed on common progenitors of retina and RPE and persisting expression following RPE differentiation (its expression diminishes in cells that progress along a retina lineage), and the RPE-specific marker RPE-65 after two weeks. The process required direct cell-cell contact between BMSC and RPE. No staining for RPE markers was detected when a membrane separated the two populations of cells. This was a specific effect, as no positive staining was detected when RPE cells were replaced with fibroblasts.[91]

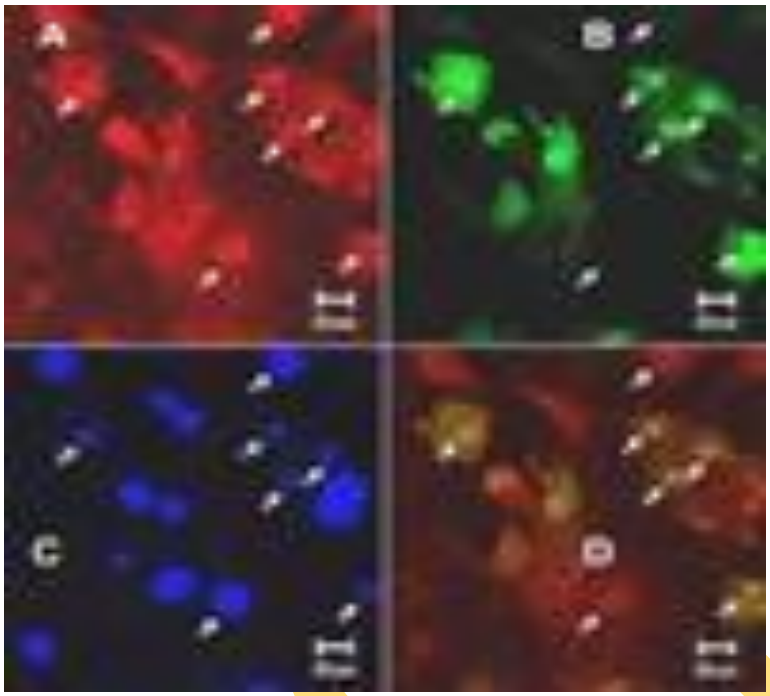

Figure 4Co-culture with RPE cells for two weeks leads to the expression of RPE-specific markers on sorted Sca-1$^+$ BMSC

Figure 5Cross section of a mouse eye six weeks after $NaIO_3$ injection and i.v. transplantation of EGFP$^+$ BMSC

Conclusions:

It is important to note that degenerations in the mammalian retina, initiated by defects in photoreceptors or RPE, often leave the neural retina deafferented. It responds to this challenge by remodelling, first by subtle changes in neuronal structure and later by large-scale reorganization and represents the invocation of mechanisms resembling developmental and CNS plasticity. This neuronal remodelling and the formation of a glial seal may abrogate many cellular and bionic rescue strategies. On the other hand, survivor neurons appear to be stable, healthy, active cells and given the evidence of their reactivity to deafferentation, it may be possible to influence their emergent rewiring and migration habits.[102]

Reference:

1. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology. 1992;99:933–943.
2. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. Prog Retin Eye Res. 2001;20:385–414.
3. Sippy BD, Hinton DR. Aging of retina and retinal pigment epithelium. In: Lim JI, editor. Age-Related macular Degeneration. New York: Marcel Dekker, Inc.; 2002. pp. 1–14.
4. Kunze C, Elsner AE, Beausencourt E, et al. Spatial extent of pigment epithelial detachments in age-related macular degeneration. Ophthalmology. 1999;106:1830–1840.
5. Shen JK, Dong A, Hackett SF, et al. Oxidative damage in age-related macular degeneration. Histol Histopathol. 2007;22:1301–1308.
6. He X, Hahn P, Iacovelli J, et al. Iron homeostasis and toxicity in retinal degeneration. Prog Retin Eye Res. 2007;26:649–673.
7. Crabb JW, Miyagi M, Gu X, et al. Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci USA. 2002;99:14682–14687.
8. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol. 2002;134:411–431.
9. Tezel TH, Bora NS, Kaplan HJ. Pathogenesis of age-related macular degeneration. Trends Mol Med. 2004;10:417–420.
10. Donoso LA, Kim D, Frost A, Callahan A, Hageman G. The role of inflammation in the pathogenesis of age-related macular degeneration. Surv Ophthalmol. 2006;51:137–152.
11. Berger AS, Kaplan HJ. Clinical experience with the surgical removal of subfoveal neovascular membranes. Short-term postoperative results. Ophthalmology. 1992;99:969–975.
12. Hsu JK, Thomas MA, Ibanez H, Green WR. Clinicopathologic studies of an eye after submacular membranectomy for choroidal neovascularization. Retina. 1995;15:43–52.
13. Nasir MA, Sugino I, Zarbin MA. Decreased choriocapillaris perfusion following surgical excision of choroidal neovascular membranes in age-related macular degeneration. Br J Ophthalmol. 1997;81:481–489.
14. Pollack JS, Del Priore LV, Smith ME, Feiner MA, Kaplan HJ. Postoperative abnormalities of the choriocapillaris in exudative age-related macular degeneration. Br J Ophthalmol. 1996;80:314–318.
15. Postelmans L, Pasteels B, Coquelet P, et al. Severe pigment epithelial alterations in the treatment area following photodynamic therapy for classic choroidal neovascularization in young females. Am J Ophthalmol. 2004;138:803–808.
16. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. Eye. 1988;2(Pt 5):552–577.
17. Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006;355:1419–1431.
18. Brown DM, Kaiser PK, Michels M, et al. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med. 2006;355:1432–1444.
19. Morris B, Imrie F, Armbrecht AM, Dhillon B. Age-related macular degeneration and recent developments: new hope for old eyes? Postgrad Med J. 2007;83:301–307.

20. Del Priore LV, Geng L, Tezel TH, Kaplan HJ. Extracellular matrix ligands promote RPE attachment to inner Bruch's membrane. Curr Eye Res. 2002;25:79–89.
21. Tezel TH, Kaplan HJ, Del Priore LV. Fate of human retinal pigment epithelial cells seeded onto layers of human Bruch's membrane. Invest Ophthalmol Vis Sci. 1999;40:467–476.
22. Gullapalli VK, Sugino IK, Van PY, Shah S, Zarbin MA. Impaired RPE survival on aged submacular human Bruch's membrane. Exp Eye Res. 2005;80:235–248.
23. Phillips SJ, Sadda SR, Tso MO, et al. Autologous transplantation of retinal pigment epithelium after mechanical debridement of Bruch's membrane. Curr Eye Res. 2003;26:81–88.
24. Sohocki MM, Daiger SP, Bowne SJ, et al. Prevalence of mutations causing retinitis pigmentosa and other inherited retinopathies. Hum Mutat. 2001;17:42–51.
25. Koenekoop RK, Lopez I, den Hollander AI, Allikmets R, Cremers FP. Genetic testing for retinal dystrophies and dysfunctions: benefits, dilemmas and solutions. Clin Exp Ophthalmol. 2007;35:473–485.
26. Hamel C. Retinitis pigmentosa. Orphanet J Rare Dis. 2006;1:40.
27. Ahmad I. Stem cells: new opportunities to treat eye diseases. Invest Ophthalmol Vis Sci. 2001;42:2743–2748.
28. Sagdullaev BT, Aramant RB, Seiler MJ, Woch G, McCall MA. Retinal transplantation-induced recovery of retinotectal visual function in a rodent model of retinitis pigmentosa. Invest Ophthalmol Vis Sci. 2003;44:1686–1695.
29. Tschernutter M, Schlichtenbrede FC, Howe S, et al. Long-term preservation of retinal function in the RCS rat model of retinitis pigmentosa following lentivirus-mediated gene therapy. Gene Ther. 2005;12:694–701.
30. Wu WC, Lai CC, Chen SL, et al. Gene therapy for detached retina by adeno-associated virus vector expressing glial cell line-derived neurotrophic factor. Invest Ophthalmol Vis Sci. 2002;43:3480–3488.
31. Sieving PA, Caruso RC, Tao W, et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc Natl Acad Sci USA. 2006;103:3896–3901.
32. Perry D. Patients' voices: the powerful sound in the stem cell debate. Science. 2000;287:1423.
33. Marshak DR, Gottleib D, Gardner RL. Stem Cell Biology. In: Marshak DR, Gottleib D, Gardner RL, editors. Stem Cell Biology. Cold Spring Harbor, NY: CSHL Press; 2000. pp. 1–16.
34. Haruta M. Embryonic stem cells: potential source for ocular repair. Semin Ophthalmol. 2005;20:17–23.
35. Swijnenburg RJ, Tanaka M, Vogel H, et al. Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium. Circulation. 2005;112:I166–I172.
36. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell. 2008;132:567–582.
37. Lavik EB, Klassen H, Warfvinge K, Langer R, Young MJ. Fabrication of degradable polymer scaffolds to direct the integration and differentiation of retinal progenitors. Biomaterials. 2005;26:3187–3196.
38. Shamblott MJ, Axelman J, Wang S, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc Natl Acad Sci USA. 1998;95:13726–13731.
39. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145–1147.

40. Hoffman LM, Carpenter MK. Characterization and culture of human embryonic stem cells. Nat Biotechnol. 2005;23:699–708.
41. Arnhold S, Lenartz D, Kruttwig K, et al. Differentiation of green fluorescent protein-labeled embryonic stem cell-derived neural precursor cells into Thy-1-positive neurons and glia after transplantation into adult rat striatum. J Neurosurg. 2000;93:1026–1032.
42. Brustle O, Jones KN, Learish RD, et al. Embryonic stem cell-derived glial precursors: a source of myelinating transplants. Science. 1999;285:754–756.
43. Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R. Endothelial cells derived from human embryonic stem cells. Proc Natl Acad Sci USA. 2002;99:4391–4396.
44. Vodyanik MA, Bork JA, Thomson JA, Slukvin II. Human embryonic stem cellderived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. Blood. 2005;105:617–626.
45. Xu C, Police S, Rao N, Carpenter MK. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circ Res. 2002;91:501–508.
46. Rambhatla L, Chiu CP, Kundu P, Peng Y, Carpenter MK. Generation of hepatocyte-like cells from human embryonic stem cells. Cell Transplant. 2003;12:1–11.
47. Winkler J, Hescheler J, Sachinidis A. Embryonic stem cells for basic research and potential clinical applications in cardiology. Biochim Biophys Acta. 2005;1740:240–248.
48. Meyer JR. Human embryonic stem cells and respect for life. J Med Ethics. 2000;26:166–170.
49. Anderson DJ, Gage FH, Weissman IL. Can stem cells cross lineage boundaries? Nat Med. 2001;7:393–395.
50. Lin H. The tao of stem cells in the germline. Annu Rev Genet. 1997;31:455–491.
51. La PC. Cancer Stem Cells: Lessons From Melanoma. Stem Cell Rev. 2008
52. Asahara T, Kalka C, Isner JM. Stem cell therapy and gene transfer for regeneration. Gene Ther. 2000;7:451–457.
53. Thomas ED, Storb R, Clift RA, et al. Bone marrow transplantation (Second of Two Parts) N Engl J Med. 1975;292:895–902.
54. Ratajczak MZ, Kucia M, Reca R, et al. Stem cell plasticity revisited: CXCR4-positive cells expressing mRNA for early muscle, liver and neural cells 'hide out' in the bone marrow. Leukemia. 2004;18:29–40.
55. Kucia M, Ratajczak J, Reca R, Janowska-Wieczorek A, Ratajczak MZ. Tissue-specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury. Blood Cells Mol Dis. 2004;32:52–57.
56. Caplice NM, Bunch TJ, Stalboerger PG, et al. Smooth muscle cells in human coronary atherosclerosis can originate from cells administered at marrow transplantation. Proc Natl Acad Sci USA. 2003;100:4754–4759.
57. Forbes SJ, Poulsom R, Wright NA. Hepatic and renal differentiation from blood-borne stem cells. Gene Ther. 2002;9:625–630.
58. Krause DS, Theise ND, Collector MI, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell. 2001;105:369–377.
59. Mezey E, Key S, Vogelsang G, et al. Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci USA. 2003;100:1364–1369.

60. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature. 2001;410:701–705.
61. Clarke DL, Johansson CB, Wilbertz J, et al. Generalized potential of adult neural stem cells. Science. 2000;288:1660–1663.
62. McKay R. Stem cells in the central nervous system. Science. 1997;276:66–71.
63. Tropepe V, Coles BL, Chiasson BJ, et al. Retinal stem cells in the adult mammalian eye. Science. 2000;287:2032–2036.
64. Oh H, Bradfute SB, Gallardo TD, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci USA. 2003;100:12313–12318.
65. Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotechnol. 2003;21:763–770.
66. Reca R, Mastellos D, Majka M, et al. Functional receptor for C3a anaphylatoxin is expressed by normal hematopoietic stem/progenitor cells, and C3a enhances their homing-related responses to SDF-1. Blood. 2003;101:3784–3793.
67. Tsonis PA. Regenerative biology: the emerging field of tissue repair and restoration. Differentiation. 2002;70:397–409.
68. Dowell JD, Rubart M, Pasumarthi KB, Soonpaa MH, Field LJ. Myocyte and myogenic stem cell transplantation in the heart. Cardiovasc Res. 2003;58:336–350.
69. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA. 2001;98:10344–10349.
70. Kolb HJ, Guenther W, Gyurkocza B, et al. Tolerance and chimerism. Transplantation. 2003;75:26S–31S.
71. Terada N, Hamazaki T, Oka M, et al. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature. 2002;416:542–545.
72. Ying QL, Nichols J, Evans EP, Smith AG. Changing potency by spontaneous fusion. Nature. 2002;416:545–548.
73. Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature. 2003;425:968–973.
74. Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ. Hematopoietic stem cells convert into liver cells within days without fusion. Nat Cell Biol. 2004;6:532–539.
75. Marin-Garcia J, Goldenthal MJ. Application of stem cells in cardiology: where we are and where we are going. Curr Stem Cell Res Ther. 2006;1:1–11.
76. Xu YQ, Liu ZC. Therapeutic Potential of Adult Bone Marrow Stem Cells in Liver Disease and Delivery Approaches. Stem Cell Rev. 2008
77. Lowry NA, Temple S. Making human neurons from stem cells after spinal cord injury. PLoS Med. 2007;4:236–238.
78. Ramachandran AC, Bartlett LE, Mendez IM. A multiple target neural transplantation strategy for Parkinson's disease. Rev Neurosci. 2002;13:243–256.
79. Slavin S, Kurkalli BG, Karussis D. The potential use of adult stem cells for the treatment of multiple sclerosis and other neurodegenerative disorders. Clin Neurol Neurosurg. 2008
80. Lund RD, Kwan AS, Keegan DJ, et al. Cell transplantation as a treatment for retinal disease. Prog Retin Eye Res. 2001;20:415–449.

81. Ahmad I, Tang L, Pham H. Identification of neural progenitors in the adult mammalian eye. Biochem Biophys Res Commun. 2000;270:517–521.
82. Coles BL, Angenieux B, Inoue T, et al. Facile isolation and the characterization of human retinal stem cells. Proc Natl Acad Sci USA. 2004;101:15772–15777.
83. MacLaren RE, Pearson RA, MacNeil A, et al. Retinal repair by transplantation of photoreceptor precursors. Nature. 2006;444:203–207.
84. West EL, Pearson RA, Tschernutter M, et al. Pharmacological disruption of the outer limiting membrane leads to increased retinal integration of transplanted photoreceptor precursors. Exp Eye Res. 2008;86:601–611.
85. Dong X, Pulido JS, Qu T, Sugaya K. Differentiation of human neural stem cells into retinal cells. Neuroreport. 2003;14:143–146.
86. Giordano F, De MA, Vetrini F, Marigo V. Fibroblast growth factor and epidermal growth factor differently affect differentiation of murine retinal stem cells in vitro. Mol Vis. 2007;13:1842–1850.
87. Angenieux B, Schorderet DF, Arsenijevic Y. Epidermal growth factor is a neuronal differentiation factor for retinal stem cells in vitro. Stem Cells. 2006;24:696–706.
88. Brabletz T, Hlubek F, Spaderna S, et al. Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. Cells Tissues Organs. 2005;179:56–65.
89. Hossain MA, Russell JC, Gomez R, Laterra J. Neuroprotection by scatter factor/hepatocyte growth factor and FGF-1 in cerebellar granule neurons is phosphatidylinositol 3-kinase/akt-dependent and MAPK/CREB-independent. J Neurochem. 2002;81:365–378.
90. Korte GE, Reppucci V, Henkind P. RPE destruction causes choriocapillary atrophy. Invest Ophthalmol Vis Sci. 1984;25:1135–1145.
91. Li Y, Atmaca-Sonmez P, Schanie CL, et al. Endogenous Bone Marrow Derived Cells Express Retinal Pigment Epithelium Cell Markers and Migrate to Focal Areas of RPE Damage. Invest Ophthalmol Vis Sci. 2007;48:4321–4327.
92. Enzmann V, Row BW, Yamauchi Y, et al. Behavioral and anatomical abnormalities in a sodium iodate-induced model of retinal pigment epithelium degeneration. Exp Eye Res. 2006;82:441–448.
93. Kahn J, Byk T, Jansson-Sjostrand L, et al. Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. Blood. 2004;103:2942–2949.
94. Li Y, Reca R, Sonmez P, et al. Retinal pigment epithelium damage enhances expression of chemoattracts and migration of bone marrow-derived stem cells. Invest Ophthalmol Vis Sci. 2006;47:1646–1652.
95. Atmaca-Sonmez P, Li Y, Yamauchi Y, et al. Systemically transferred hematopoietic stem cells home to the subretinal space and express RPE-65 in a mouse model of retinal pigment epithelium damage. Exp Eye Res. 2006;83:1295–1302.
96. Anderson DH, Guerin CJ, Erickson PA, Stern WH, Fisher SK. Morphological recovery in the reattached retina. Invest Ophthalmol Vis Sci. 1986;27:168–183.
97. Chiou SH, Kao CL, Peng CH, et al. A novel in vitro retinal differentiation model by co-culturing adult human bone marrow stem cells with retinal pigmented epithelium cells. Biochem Biophys Res Commun. 2005;326:578–585.
98. Haruta M, Sasai Y, Kawasaki H, et al. In vitro and in vivo characterization of pigment epithelial cells differentiated from primate embryonic stem cells. Invest Ophthalmol Vis Sci. 2004;45:1020–1025.

99. Kucia M, Reca R, Jala VR, et al. Bone marrow as a home of heterogenous populations of nonhematopoietic stem cells. Leukemia. 2005;19:1118–1127.
100. Kucia M, Zuba-Surma EK, Wysoczynski M, et al. Adult marrow-derived very small embryonic-like stem cells and tissue engineering. Expert Opin Biol Ther. 2007;7:1499–1514.
101. Kucia M, Wojakowski W, Reca R, et al. The migration of bone marrow-derived non-hematopoietic tissue-committed stem cells is regulated in an SDF-1-, HGF-, and LIF-dependent manner. Arch Immunol Ther Exp (Warsz) 2006;54:121–135.
102. Marc RE, Jones BW, Watt CB, Strettoi E. Neural remodeling in retinal degeneration. Prog Retin Eye Res. 2003;22:607–655.
103. Tabata Y. Current status of regenerative medical therapy based on drug delivery technology. Reprod Biomed Online. 2008;16:70–80.
104. Dawn B, Guo Y, Rezazadeh A, et al. Postinfarct cytokine therapy regenerates cardiac tissue and improves left ventricular function. Circ Res. 2006;98:1098–1105.
105. Petit I, Szyper-Kravitz M, Nagler A, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol. 2002;3:687–694.
106. Neipp M, Zorina T, Domenick MA, Exner BG, Ildstad ST. Effect of FLT3 ligand and granulocyte colony-stimulating factor on expansion and mobilization of facilitating cells and hematopoietic stem cells in mice: kinetics and repopulating potential. Blood. 1998;92:3177–3188.
107. Pituch-Noworolska A, Majka M, Janowska-Wieczorek A, et al. Circulating CXCR4-positive stem/progenitor cells compete for SDF-1-positive niches in bone marrow, muscle and neural tissues: an alternative hypothesis to stem cell plasticity. Folia Histochem Cytobiol. 2003;41:13–21.
108. Singec I, Jandial R, Crain A, Nikkhah G, Snyder EY. The leading edge of stem cell therapeutics. Annu Rev Med. 2007;58:313–328.
109. Sharp J, Keirstead HS. Therapeutic applications of oligodendrocyte precursors derived from human embryonic stem cells. Curr Opin Biotechnol. 2007;18:434–440.