Retrovirus-mediated transduction of a cytosine deaminase gene preserves the stemness of mesenchymal stem cells

Jin Sung Park 1,2,7, Da-Young Chang 1,7, Ji-Hoi Kim 1,2, Jin Hwa Jung 1, JoonSeong Park 3, Se-Hyuk Kim 4, Young-Don Lee 1,5, Sung-Soo Kim 1,5 and Haeyoung Suh-Kim 1,3,6

Human mesenchymal stem cells (MSCs) have emerged as attractive cellular vehicles to deliver therapeutic genes for ex-vivo therapy of diverse diseases; this is, in part, because they have the capability to migrate into tumor or lesion sites. Previously, we showed that MSCs could be utilized to deliver a bacterial cytosine deaminase (CD) suicide gene to brain tumors. Here we assessed whether transduction with a retroviral vector encoding CD gene altered the stem cell property of MSCs. MSCs were transduced at passage 1 and cultivated up to passage 11. We found that proliferation and differentiation potentials, chromosomal stability and surface antigenicity of MSCs were not altered by retroviral transduction. The results indicate that retroviral vectors can be safely utilized for delivery of suicide genes to MSCs for ex-vivo therapy. We also found that a single retroviral transduction was sufficient for sustainable expression up to passage 10. The persistent expression of the transduced gene indicates that transduced MSCs provide a tractable and manageable approach for potential use in allogeneic transplantation.

Keywords: ex-vivo therapy; gene therapy; mesenchymal stem cell; retrovirus; safety; suicide gene

INTRODUCTION

Mesenchymal stem cells (MSCs) have been utilized for the treatment of diverse diseases, including neuropathies such as Parkinson’s disease,1 Huntington’s disease,2 multiple sclerosis,3,4 amyotrophic lateral sclerosis,5 ischemic stroke,5,7 and non-neurological diseases such as myocardial infarction,6,9 and graft-versus-host diseases.10 The therapeutic effects of MSCs are ascribed to their paracrine functions that include the secretion of beneficial molecules,11,12 anti-inflammatory factors,13,14 or extracellular matrix.15 However, a major challenge is how to render MSCs more disease-specific and enhance their paracrine effects. As MSCs are highly migratory to lesion and tumor sites,16 it has been suggested they can be used as cellular vehicles to deliver therapeutic genes to target tissues for ex-vivo therapy and to overcome targeting problems of conventional gene therapy. To tailor MSCs to be more disease-specific or to modify them as gene carriers, viral vectors are frequently utilized to introduce therapeutic genes into MSCs.

Previously, we showed that MSCs could be utilized as a cellular vehicle to deliver a cytosine deaminase (CD) gene to brain tumors.17 CD genes are naturally expressed in bacteria and fungi, but absent in humans. CD can convert a nontoxic prodrug, 5-fluorocytosine (5-FC) into 5-fluorouracil, an anti-cancer drug that has been used for the treatment of gastrointestinal cancers.18 Cell membranes are highly permeable to 5-fluorouracil, which can enter neighboring cells through simple diffusion and exert cytotoxic effects by interfering with DNA and RNA synthesis (bystander effects). We showed that MSCs infected with a retroviral vector expressing an Escherichia coli CD gene could migrate toward brain tumors and suppress tumor growth through bystander
Retroviral transduction of MSC
JS Park et al

MATERIALS AND METHODS
Isolation and cultivation of MSCs
Human MSCs were originally derived from the iliac crest bone marrow of healthy 10- to 15-year-old donors undergoing bone-marrow aspiration for future allogenic transplantation, with approval of the Institutional Review Board of Ajou University, Medical Center, as previously described. Briefly, mononucleate adherent cells were collected and maintained in Dulbecco's modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin (Invitrogen, Grand Island, NY, USA) and 10 ng ml⁻¹ basic fibroblast growth factor (Dong-A Pharmaceutical Co., Youngin, Korea).

Retroviral transduction
CD-expressing MSCs were prepared by transducing MSCs with a retroviral vector encoding CD, as previously described. The CD gene was cloned from E. coli K12 MG1655 (KRRIBB, Daejon, Korea) by PCR (forward primer: 5'-GAA TTC AGG CTA GCA ATG TCG AAT AAC 3'; reverse primer: 5'-GGA TCC TCT AGC TGG CAG AC A GCC GC-3') and then into a pFIP plasmid (ViroMed, Seoul, Korea). A retroviral vector containing pFIP/CD was produced in a FLYRD18-packaging cell line expressing the Moloney murine leukemia virus gag-pol gene and the cat endogenous virus RD114 env gene. Two days after plating of packaging cells in a density of 1.5 × 10⁹/T-75 flask, the viral supernatants were collected and syringe-filtered using a 0.45-μm filter. MSCs at passage 1 were plated at a density of 1 × 10⁵ cell per 100 mm dish and exposed to retroviruses with 20 multiplicity of infection for 8 h in the presence of 4 μg ml⁻¹ polybrene (Sigma-Aldrich, St Louis, MO, USA) and 10 ng ml⁻¹ basic fibroblast growth factor. Two days later, cells were subcultured and 2 μg ml⁻¹ puromycin (Sigma-Aldrich) was added to the culture for 2 weeks. Surviving cells were pooled and maintained by subculturing every 5-7 days. To compare growth kinetics of MSCs with MSC/CD, cells were counted by trypan blue exclusion test and plated in a density of 1000 cells per cm² for the next passage in culture. All cell culture medium was replaced with fresh one every 2 or 3 days.

Differentiation
Adipogenic, osteogenic, and chondrogenic differentiation were performed as previously described with a slight modification. Briefly, cells were plated at a density of 2 × 10⁴ cells per 3.8 cm² in a culture medium and grown to confluence. The culture medium was replaced with adipogenic medium supplemented with 0.5 mm isobutylmethylxanthine, 60 μM indomethacine, 1 μM dexamethasone, 10 μg ml⁻¹ of insulin for 2 weeks, or osteogenic medium supplemented with 0.1 μM dexamethasone, 60 μM ascorbic acid and 10 μM β-glycerophosphate for 5 weeks. Adipogenic differentiation was verified by accumulation of lipid droplets stained with Oil Red O; osteogenic differentiation and the associated accumulation of extracellular calcium crystals were scored by staining by Alizarin Red S. Chondrogenic differentiation was induced by cultivating 2 × 10⁵ cells in pellets in an induction medium supplemented with 1% fetal bovine serum, 6.25 μg ml⁻¹ insulin, 10 ng ml⁻¹ transforming growth factor beta 1 (TGF-β1) and 50 ng ml⁻¹ ascorbic acid for 6 weeks. Alcian blue was used to stain metachromatic extracellular material in the pellet, and then, Nuclear Fast Red was used for counter staining of chondrocytes.

Flow cytometry analysis
To measure the expression of surface antigen, MSCs or CD-expressing MSCs were collected with 0.25% Trypsin/EDTA (Invitrogen) and resuspended in phosphate-buffered saline (PBS) containing 1% bovine
serum albumin. Cells were stained with fluorochrome-conjugated antibodies against STRO-1, HLA-ABC, HLA-DR, CD34, CD45, CD90, CD105, CD11b, CD29, CD49a, CD73, CD117 and isotype controls (Biolegend, San Diego, CA, USA) for 10 min at room temperature (RT). After washing with PBS containing 1% bovine serum albumin, cells were analyzed using BD FACS vantage (BD Biosciences, San Jose, CA, USA) with CellQuestPro software (BD Biosciences). All assays included isotype controls.

**Anti-cancer effects**

For *in-vitro* suicide effects, cells were plated at a density of 10,000 cells per well in 12-well plates, and 24 h later, 5-FC (Archimica, Flintshire, UK) was added at the indicated concentrations. MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma-Aldrich) assays were performed to measure cell viability on day 7. The medium was replaced every 2 days with fresh growth medium containing the indicated concentrations of 5-FC. The values at each 5-FC concentration are expressed relative to those of untreated cells and presented as the means ± s.e. To assess bystander effects *in vitro*, U87MG glioma cells were transduced by a lentiviral vector expressing green fluorescent protein (GFP), and GFP-positive cells were sorted by fluorescence-activated cell sorting. U87MG/GFP cells were cocultured with MSCs or MSC/CD cells at a ratio of 10:1 in 12-well plates. Twenty-four hours later, 5-FC was added to obtain the indicated concentrations of 5-FC. The cells were analyzed as described above.

**Quantitative PCR**

Total RNA was isolated from cells using RNAzol B (Tel-Test, Friendswood, TX, USA) and cDNA synthesized from 1 μg of RNA using the First-strand cDNA synthesis kit (Roche, Mannheim, Germany). Amplification was performed using a Taqman universal PCR master mix kit (Applied Biosystems, Foster City, CA, USA) and cDNA synthesized from 1 μg of total RNA using the First-strand cDNA synthesis kit (Roche, Mannheim, Germany). Relative CD gene expression to glyceraldehyde-3-phosphate dehydrogenase was measured with anti-CD antibody (1:500) for 30 min at RT, and then with Alexa 488-conjugated anti-rabbit IgG antibody. After washing twice, cells were analyzed as described above.

**Chromosomal stability and tumorigenicity tests**

For chromosome analysis, 20 cells at metaphase were counted after staining with Leishman stain solution, and 5 cells were analyzed for more detailed karyotyping using CytoVision (Applied Imaging International, San Jose, CA, USA). GTG-banding (G-bandning by trypsin and Giemsa stain) with 450 bands of resolution was conducted in a clinical Cytogenetics Laboratory at Ajou University Hospital (http://www.ajoumc.or.kr). To measure *in-vivo* transformation, MSC, MSC/CD and U87MG cells were cultured in soft agar by coculturing with MSCs or MSC/CD cells at a ratio of 10:1 in 12-well plates. Twenty-four hours later, 5-FC was added to obtain the indicated concentrations of 5-FC. The medium was replaced every 2 days with fresh growth medium containing the indicated concentrations of 5-FC. The values at each 5-FC concentration are expressed relative to those of untreated cells and presented as the means ± s.e.). PCR primers and probes are summarized in Table 1.

## Table 1 Primers and probes for quantitative PCR

| Gene   | Sequences                                                                 |
|--------|---------------------------------------------------------------------------|
| CD     | Forward 5'-TGATGAGATCGATGACGACAGTC-3'                                     |
| Reverse| 5'-GGGTTGCGACAAATAGTAACCGG-3'                                            |
| Probe  | 56-FAM/5'-TATACCTCA/ZNCGCTGTTCCGCTGAAA-3'/3IABkFQ                         |
| GAPDH  | Forward 5'-GGCCCATCCACAGTCCTCTG-3'                                       |
| Reverse| 5'-CAGGTCCTGAAGTCATCGAGCA-3'                                             |
| Probe  | 56-FAM/3'-ATGCCACAAZ/ZNCGTCGACATTAC-3'/3IABkFQ                          |

Abbreviations: CD, cytokine deaminase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**RESULTS**

Retroviral transduction of MSCs

MSCs were isolated from the bone marrow of a human volunteer and expanded *in vitro* as previously reported.

---

**Table 1 Primers and probes for quantitative PCR**

| Gene | Sequences |
|------|-----------|
| CD   | Forward 5'-TGATGAGATCGATGACGACAGTC-3' |
|      | Reverse 5'-GGGTTGCGACAAATAGTAACCGG-3' |
|      | Probe 56-FAM/5'-TATACCTCA/ZNCGCTGTTCCGCTGAAA-3'/3IABkFQ |
| GAPDH | Forward 5'-GGCCCATCCACAGTCCTCTG-3' |
|      | Reverse 5'-CAGGTCCTGAAGTCATCGAGCA-3' |
|      | Probe 56-FAM/3'-ATGCCACAAZ/ZNCGTCGACATTAC-3'/3IABkFQ |

**Abbreviations:** CD, cytokine deaminase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
Retroviral vectors expressing the E. coli CD gene generated in FLYRD18 packaging cells were added to MSCs at passage 1, and CD-expressing MSCs (MSC/CD) were selected in the presence of puromycin. Initially, the growth rate of MSC/CD cells was temporarily retarded by transduction and selection, but was recovered immediately (Figure 1a). Once they were grown to confluence at day 7, MSCs and MSC/CD cells were exponentially expanded by approximately 19-fold per every 6-day culture (Figure 1b); the growth rates were indistinguishable up to passage 10 between both cell types. These data indicate that neither transduction with retroviral vectors nor CD expression interferes with the proliferation capability of MSCs.

Characterization of MSC/CD cells
According to the International Society for Cellular Therapy, MSCs can be defined by three criteria, such as adhering to plastic, expressing specific surface antigens and differentiating into mesodermal multilineages. Both naïve MSCs and MSCs/CD adhered to plastic culture dishes on which they exhibited similar fibroblastic morphology (Figure 2a). Both cell types retained differentiation potential when induced to differentiate into adipocytes, osteocytes and chondrocytes (Figure 2a). These MSCs and MSCs/CD cells both expressed the typical surface antigen of classical MSCs. Specifically, they were positive for STRO-1, HLA-ABC, CD29, CD49a, CD73, CD90 and CD105, whereas they were negative for HLA-DR, CD45, CD34, CD11b and CD117 (Figure 2b). These results indicate that retrovirus-mediated CD expression does not alter the morphology, multipotency and surface antigenic properties of MSCs.

In vitro anti-cancer effects of MSC/CD
Suicide effects of MSC/CD cells were measured in the presence of 5-FC. MTT assays showed that 5-FC decreased the number of MSC/CD cells with IC_{50} (half maximal inhibitory concentration) values of 60.4 μM (Figure 3a). Parental MSCs were resistant to 5-FC up to a concentration of 1 mM (open circles in Figure 3a), indicating the specificity of the suicide function of CD. The anticancer effects of MSC/CD cells were tested against cocultured U87MG glioma cells stably expressing GFP (U87MG/GFP) to distinguish glioma cells. When cocultured with parental MSCs, U87MG cells continued to proliferate even in the presence of 1 mM 5-FC (Figure 3c). In contrast, when cocultured with MSC/CD cells, the growth of U87MG/GFP cells was suppressed upon 5-FC treatment, with an IC_{50} of 32.8 μM (Figure 3b).

Long-lasting expression of CD in MSC/CD cells
Retroviral vectors integrate into the host genome and induce long-term expression of the gene. However, it is also known that the genes exogenously introduced by retroviral vector can be silenced by methylation of cytomegalovirus promoter or long terminal repeat promoter. To test whether the CD gene was persistently expressed after long-term culture, we generated a polyclonal anti-CD antibody. Immunocytochemistry indicated a specific immunoreactivity only in MSCs/CD cells, but not in MSCs (Figure 4a). Fluorescence-activated cell sorting analysis indicated that 94.7% of MSC/CD cells persistently express the CD gene at passage 8 (Figure 4b). Quantitative reverse transcription-PCR analysis indicated that MSC/CD cells ranging from passage 2 to passage 9 expressed CD mRNA at similar levels (Figure 4c). Western blot analysis indicated that the CD expression level of MSC/CD cells was similar to the level at passage 5 and passage 8 (Figure 4d). The results indicated that expression of the CD gene that was transduced at passage 1 using a retroviral vector was sustained after long-term cultivation up to passage 10, by which time the cells had expanded 10^{12}-fold.

Genetic stability of MSC/CD cells
One of the general concerns regarding retroviral vectors is that they can induce genomic instability owing to their integration
into host chromosomes or other effects they exert over long-term in-vitro culture. Although CD-expressing MSCs would be eliminated in vivo when combined with 5-FC administration, we tested the genomic stability of MSC/CD cells with a G-banding assay that could evaluate microscopic chromosomal aberrations, such as nondisjunction or translocation of chromosomes. Such as MSCs, MSCs/CD cells contained normal 22 autosomal chromosome pairs and XY (Figure 5a). MSC/CD obtained from an independent transduction with retroviral vectors also showed the normal karyotype, even after long-term cultivation in vitro (data not shown). The results indicate that retrovirus-mediated transduction or long-term cultivation does not cause microscopic chromosomal aberration. Both MSC and MSC/CD cells cultured to passage 8 failed to form any colonies in soft agar under conditions in which U87MG glioblastoma cells formed numerous colonies (Figure 5b). This suggests that unlike cancer cells, both cell types cannot grow anchorage-independently. Indeed, when both cell types were subcutaneously transplanted to nude mice, they did not form any detectable tumors over a 5-month period, whereas U87MG glioblastoma cells grew to solid tumors with a volume of $5.4 \pm 0.8$ cm³ by 53 days (Figures 5c and d).

**DISCUSSION**

MSCs are currently being evaluated for cell-based therapies of diverse tissue injury and degenerative diseases. MSCs can be obtained via relatively non-invasive methods from diverse tissues and autologously transplanted after being expanded to a large scale in vitro. Recently, therapeutic genes have been introduced to render MSCs more tissue- and disease-specific. In this study, we provide evidence that retroviral vectors can be efficiently utilized to introduce therapeutic genes into MSCs, because they allow sustainable expression of therapeutic genes without disturbing the stemness and genetic stability of MSCs during expansion in vitro.

MSCs can be identified by their fibroblastic morphology, adhering activity to plasticware and surface antigen profiles, whereas the stemness of MSCs can be defined by their capability of long-term proliferation and differentiation into mesodermal lineage cell types. We proved that our MSCs and MSC/CD cells could be cultivated as adherent cells. Both

---

Figure 2 No effects of retroviral transduction on multipotency and surface antigen profiles. (a) Both naive MSCs and MSC/CD were induced to differentiate into adipocytes, osteocytes and chondrocytes for 3, 5 and 6 weeks, and were stained by Oil Red O, Alizarin Red S and Alcian blue/Nuclear Fast Red, respectively. Both MSCs and MSC/CD cells were able to differentiate into three mesodermal lineage cells. Scale bar = 50 μm. (b) Both naive MSCs and MSC/CD cells showed the same phenotypes: positive for STRO-1, HLA-ABC, CD90, CD105, CD29, CD49a and CD73, whereas negative for all isotype controls, HLA-DR, CD11b, CD34, CD45 and CD117. CD-retrovirus transduction did not affect the surface antigenicity of MSCs.
**Figure 3** In-vitro cytotoxic effects of MSC/CD in combination with 5-FC. (a) To quantify the suicide effects, MSC/CD cells were incubated in the presence of the indicated concentrations of 5-FC for 7 days. Surviving MSC/CD cells were quantified by MTT assays. (b) To quantify the bystander effects, U87MG glioma cells stably expressing GFP (U87MG/GFP) were cocultured with MSC/CD cells in the presence of indicated concentrations of 5-FC for 7 days. The remaining U87MG/GFP cells were quantified by fluorometry or (c) by fluorescent microscopy. MSCs were used as negative controls. The values are presented as the means ± s.e. at each concentration relative to the value in the absence of 5-FC. Results from at least three independent in vitro experiments were analyzed using one-way analysis of variance followed by Tukey’s honestly significant difference post-hoc test (*P < 0.05; **P < 0.01).

**Figure 4** Sustainable CD expression in MSC/CD after long-term culture. CD expression was assessed in MSC/CD during p5–p10. (a) Immunocytochemistry with anti-CD antibody indicated CD expression in MSC/CD cells at p10. Scale bar = 50 μm. (b) Fluorescence-activated cell sorting analysis indicated that 94.7% of MSC/CD cells expressed CD at p8. (c) Real-time reverse transcription-PCR indicated similar levels of CD mRNA expression in MSC/CD at ranging from p2 to p9. (d) Western blot analysis revealed similar levels of CD proteins in MSC/CD cells at p5 and p8.
cell types equally had a fibroblastic morphology and expressed surface antigens, including STRO-1, HLA-ABC, CD29, CD49a, CD73, CD90 and CD105, but not HLA-DR, CD45, CD34, CD11b and CD117. We also showed that MSCs and MSC/CD cells could grow up to passage 10 without growth retardation. Growth of MSC/CD was temporarily delayed for the first few days because of retroviral transduction. Following selection, the growth rate of MSC/CD cells returned to that observed in naive MSCs. MSCs and MSC/CD cells retained the capability to differentiate into adipocytes, osteocytes and chondrocytes. Our results indicate that neither retrovirus-mediated CD expression nor long-term cultivation alters the stemness of MSCs. Moreover, once MSC/CD cells were selected in the presence of puromycin, the expression level of CD remained stable over multiple passages; indeed, 94–99% surviving cells at passage 8–10 expressed CD when tested by flow cytometry or immunocytochemistry.

In contrast to our study, others have reported that the stemness of MSCs can be influenced by the nature of transduced genes. For example, Ngn1, a proneural transcription factor, converted the mesodermal fate of MSCs into a neural one, and Ngn1-expressing MSCs lost the capability to differentiate into mesodermal lineage cells.31 Retroviral vectors encoding Wnt-4 or glucocorticoid-induced leucine zipper converted MSCs into pro-osteogenic progenitor cells at the expense of adipogenic capability.35,36 A tendon-specific transcription factor, scleraxis, converted MSCs into tendon progenitor cells, which failed to differentiate into chondrocytes and osteocytes.37

In-vitro expansion of MSCs is required to obtain sufficient cell numbers for cell-based therapy. As mentioned earlier, it was reported that MSCs could undergo spontaneous malignant transformation during extremely extended in vitro culture. The transformed MSCs grow like cancers in an anchorage-independent manner27 and exhibited gross chromosomal aberrations.27,28 However, most laboratories including ours have demonstrated that MSCs usually stop proliferation after passage 11 (70 days in vitro culture) and cannot grow in soft agar in an anchorage-independent manner. Moreover, we could not detect any gross chromosomal aberrations in our MSCs or MSC/CD cells. Therefore, our MSCs and MSC/CD cells below passage 11 with a normal karyotype can be considered non-tumorigenic. Indeed, these cells could not form solid tumors in nude mice. More importantly, our MSC/CD cells undergo cell death owing to the suicide effects of the CD gene product in the presence of 5-FC, whereas exerting bystander effects on the cocultured U87MG glioma cells. This effectively eliminates the tumorigenic potential of MSC/CD cells, consistent with the proposal that suicide genes are safety tools that can be used to ablate unwanted abnormal cells in vivo once the therapeutic genes have been delivered to their target site.

For practical uses, retroviral vectors may have greater advantages over adenoviral vectors with respect to...
introduction of the CD suicide gene into MSCs. This is because a single retroviral transduction is sufficient to obtain stable expression of the CD gene. Once transduced cells are selected and stored, working cell banks for clinical applications can be easily manufactured in compliance with current good manufacturing practice by simple expansion without additional transduction steps. This property may help to lower medical expenses and make this therapy more economically accessible. In comparison, adenoviral vectors allow transient expression of CD genes only for a limited time, because they remain as episomes and are diluted in proportion to the host cell proliferation. Consistent with other reports that MSCs are hypo-immunogenic,38 our MSCs and MSC/CD cells also lack HLA-DR, a major histocompatibility complex class II, which causes an immune response after allogeneic injection. The hypo-immunogenicity of MSCs together with the sustainable expression of the transduced CD genes after expansion in a large quantity further warrant the clinical application of allogeneic transplantation, although autologous transplantation is always preferable to allogeneic.

ACKNOWLEDGEMENTS

This study was supported by grants of the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A101446) (to HS-K & S-SK) and a grant (10172KFDA9933) from Korea Food & Drug Administration in 2012 (to HS-K).

1 Bouchez G, Sensebe L, Vourc'h P, Garreau L, Bodard S, Rico A et al. Partial recovery of dopaminergic pathway after graft of adult mesenchymal stem cells in a rat model of Parkinson's disease. Neurochem Int 2008; 52: 1332–1342.

2 Armstrong RJ, Watts C, Svendsen CN, Dunnett SB, Rossor AE. Survival, neuronal differentiation, and fiber outgrowth of propagated human neural precursor grafts in an animal model of Huntington's disease. Cell Transplant 2000; 9: 55–64.

3 Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gwoda-Kurkalli B, Gomori JM, Kassis I et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol 2010; 67: 1187–1194.

4 Liang J, Zhang H, Hua B, Wang H, Wang J, Han Z et al. Allogeneic mesenchymal stem cells transplantation in treatment of multiple sclerosis. Mult Scler 2009; 15: 644–646.

5 Choi CI, Lee YD, Kim H, Kim SH, Suh-Kim H, Kim SS. Neural induction with neurogenin1 enhances the therapeutic potential of mesenchymal stem cells in an ALS Mouse model. Cell Transplant 2012 (e-pub ahead of print 2 April 2012; doi: 10.3727/096368912X637019).

6 Perasso L, Cogo CE, Giunti D, Gandolfo C, Ruggieri P, Uccelli A et al. Systemic administration of mesenchymal stem cells increases neuron survival after global cerebral ischemia in vivo (2VO). Neural Plast 2010 (e-pub ahead of print 19 December 2010; doi:10.1155/2010/534925).

7 Yoo SW, Kim SS, Lee SY, Lee HS, Kim HS, Lee YD et al. Mesenchymal stem cells promote proliferation of endogenous neural stem cells and survival of newborn cells in a rat stroke model. Exp Mol Med 2008; 40: 387.

8 Amado LC, Saliaris AP, Schuleri ST KH, John M, Xie JS, Cattaneo S et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA 2005; 102: 11474–11479.

9 Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 2006; 12: 459–465.

10 Le Blanc K, Rasmusson I, Sundberg B, Goetherstrom C, Hassan M, Uzunel M et al. Treatment of severe acute graft-versus-host disease with third party haploidential mesenchymal stem cells. Lancet 2004; 363: 1439–1441.

11 Tate CC, Fonck C, McGrogan M, Case CC. Human mesenchymal stromal cells and their derivative, SB623 cells, rescue neural cells via trophic support following in vitro ischemia. Cell Transplant 2010; 19: 973–984.

12 Bai L, Lennon DP, Caplan AI, Dechant A, Hecker J, Kranso J et al. Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci 2012; 15: 862–870.

13 Amado LC, Saliaris AP, Schuleri ST KH, John M, Xie JS, Cattaneo S et al. Human mesenchymal mesenchymal stem cells. Transplant Proc 2011; 43: 639–643.

14 Aizman I, Tate CC, McGrogan M, Case CC. Extracellular matrix produced by bone marrow stromal cells and by their derivative, SB623 cells, supports neural cell growth. J Neurosci 2009; 39: 3198–3206.

15 Lee DH, Ahn Y, Kim SU, Wang KC, Cho BK, Phi JH et al. Targeting rat brainstem glioma using human neural stem cells and human mesenchymal stem cells. Clin Cancer Res 2009; 15: 4925–4934.

16 Chang DY, Yoo SW, Hong SR, S Kim SJ, Yoon SH et al. The growth of brain tumors can be suppressed by multiple transplantation of mesenchymal stem cells expressing cytotoxic deaminase. Int J Cancer 2010; 127: 1975–1983.

17 Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003; 3: 330–338.

18 Ryu CH, Park SH, Park SA, Kim SM, Lim JY, Jeong CH et al. Gene therapy of intracranial glioma using interleukin 12-secreting human umbilical cord blood-derived mesenchymal stem cells. Hum Gene Ther 2011; 22: 733–743.

19 Uchibori R, Okada T, Ito T, Urabe M, Mizukami H, Kume A et al. Retroviral vector-producing mesenchymal stem cells for targeted suicide cancer gene therapy. J Gene Med 2009; 11: 373–381.

20 Sasportas LS, Kasmier R, Wakimoto H, Hingtgnen S, van de Water JA, Mohapatra G et al. Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. Proc Natl Acad Sci USA 2009; 106: 4822–4827.

21 Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res 2005; 65: 3307–3318.

22 Hacein-Bey-Ahina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 2010; 363: 355–364.

23 Ramos CA, Aspera Z, Liu E, Yoon E, Heslop HE, Rooney CM et al. An inducible caspase 9 suicide gene to improve the safety of mesenchymal stem cell therapies. Stem Cells 2010; 28: 1107–1115.

24 Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 2005; 7: 393–395.

25 Achille V, Mantelli M, Arigo G, Novara F, Avanzini MA, Bernardo ME et al. Cell-cycle phases and genetic profile of bone marrow-derived mesenchymal stromal cells expanded in vitro from healthy donors. J Cell Biochem 2011; 111: 1817–1821.

26 Rosland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H et al. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Res 2009; 69: 5331–5339.

27 Josee C, Schoemans R, Nielsen NA, Delaunoit M, Hellin AC, Heres C et al. Systematic chromosomal aberrations found in murine bone marrow-derived mesenchymal stem cells. Stem Cells Dev 2010; 19: 1167–1173.

28 Kim SS, Choi JM, Kim JW, Ham DS, Shih SL, Kim MK et al. CAMP induces neuronal differentiation of mesenchymal stem cells via activation of extracellular signal-regulated kinase/MAPK. Neuroreport 2005; 16: 1357–1361.

29 Pittenger MF. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143–147.

30 Kim SS, Yoo SW, Park TS, Ahn SC, Jeong HS, Kim JW et al. Neural induction with neurogenin1 increases the therapeutic effects of
mesenchymal stem cells in the ischemic brain. Stem Cells 2008; 26: 2217–2228.
32 Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315–317.
33 Stevanato L, Corteling RL, Stroemer P, Hope A, Heward J, Miljan EA et al. c-MycERTAM transgene silencing in a genetically modified human neural stem cell line implanted into MCAo rodent brain. BMC Neurosci 2009; 10: 86.
34 Stein S, Ott MG, Schultz-Strasser S, Jauch A, Burwinkel B, Kinner A et al. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med 2010; 16: 198–204.
35 Chang J, Sonoyama W, Wang Z, Jin Q, Zhang C, Krebsbach PH et al. Noncanonical Wnt-4 signaling enhances bone regeneration of mesenchymal stem cells in craniofacial defects through activation of p38 MAPK. J Biol Chem 2007; 282: 30938–30948.
36 Zhang W, Yang N, Shi XM. Regulation of mesenchymal stem cell osteogenic differentiation by glucocorticoid-induced leucine zipper (GILZ). J Biol Chem 2008; 283: 4723–4729.
37 Alberton P, Popov C, Pragert M, Kohler J, Shukunami C, Schieker M et al. Conversion of human bone marrow-derived mesenchymal stem cells into tendon progenitor cells by ectopic expression of scleraxis. Stem Cells Dev 2012; 21: 846–858.
38 Koppula PR, Chelluri LK, Polisetti N, Vemuganti GK. Histocompatibility testing of cultivated human bone marrow stromal cells—a promising step towards pre-clinical screening for allogeneic stem cell therapy. Cell Immunol 2009; 259: 61–65.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/3.0/