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Human Multipotent Stromal Cells (MSCs) Increase Neurogenesis and Decrease Atrophy of the Striatum in a Transgenic Mouse Model for Huntington’s Disease

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Abstract

Background: Implantation of human multipotent stromal cells from bone marrow (hMSCs) into the dentate gyrus of the hippocampus of mice was previously shown to stimulate proliferation, migration and neural differentiation of endogenous neural stem cells. We hypothesized that hMSCs would be beneficial in a mouse model of Huntington disease (HD) due to these neurogenic effects.

Results: We implanted hMSCs into the striatum of transgenic mice (N171-82Q) that are a model for HD. The implanted hMSCs rapidly disappeared over 3 to 15 days. However, they increased proliferation and neural differentiation of endogenous neural stem cells for up to 30 days. They also increased neurotrophic signaling and decreased atrophy of the striatum in 3-month old HD mice implanted with hMSCs one month earlier.

Conclusions: The results therefore suggested that neural implantation of hMSCs may be of benefit in HD but a number of parameters of dose, treatment schedule, and route of administration need to be optimized.

Introduction

HD is an autosomal dominant genetic disorder caused by the expansion of polyglutamine expeats in Exon 1 of the IT15 gene encoding for Huntingtin (Htt). The polyglutamine repeat length determines the age of onset and the overall level of function, but not the severity of the disease [1]. Although the exact mechanism underlying HD disease progression remains uncertain, the hallmark of this disease is gross atrophy of the striatum and cortex [2]. The average age of onset for HD is 37–38 years [3] and the median life expectancy after disease onset is 16.2 years (with a span of 2–45 years). The disease duration is similar regardless of age of onset [4]. HD is an extremely debilitating disease, affecting motor, cognition, and psychological stability [5]. According to the 2007 World Congress on HD, there are several clinical trials for treatments for HD, but there is currently no effective therapy [http://www.hda.org.uk/congress/index.html].

One strategy for therapy of HD is to enhance neurogenesis. Neurogenesis is not negatively affected by mutant Htt; in fact, the number of proliferating progenitor cells and new neurons in the subventricular zone (SVZ) and surrounding area was increased in HD patients compared to control [6,7,8,9]. Similarly in the HD rat model created by quinolinic acid injection, neostriatal neurogenesis was increased and neuroblasts migrated to the site of the lesion in the striatum instead of to the olfactory bulb [10]. A transgenic mouse model for HD also suggested an upregulation of neurogenesis, but there was no improvement in the HD phenotype [11]. This natural upregulation of neurogenesis may be further augmented by administration of fibroblast growth factor (FGF-2) [12,13], nerve growth factor (NGF) [14,15], and ciliary neurotrophic factor (CNTF) [16], thus improving the pathology and/or phenotype of the disease.

One potential means of increasing neurogenesis in HD is the administration of stem/progenitor cells. Among the stem/progenitor cells currently being tested are the adult stem/progenitor cells from bone marrow referred to initially as fibroblastic colony forming units, then as marrow stromal cells, subsequently as mesenchymal stem cells, and most recently as multipotent mesenchymal stromal cells, or MSCs [17,18]. MSCs are readily obtained from small samples of bone marrow and expanded in culture. They initially attracted interest as therapeutic agents because they differentiate into multiple phenotypes in culture and in vivo [19,20]. Initial observations indicated that MSCs infused into the lateral ventricles of newborn or fetal rodents engrafted and probably differentiated into neural cells [21,22,23]. Subsequent observations, particularly involving adult animals, indicated that differentiation into neural cells was unlikely to be a major factor [24,25,26]. Cell fusion and trans-differentiation may be possible; however, it is unlikely that these completely account for the therapeutic effects accounted to MSCs [25]. Instead, the cells promote repair of the CNS by creating a more favorable environment, which enhances endogenous neurogenesis.

Abstract

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environment for neuroprotection or regeneration through the secretion of cytokines and chemokines or other mechanisms [27,28,29]. In the immunodeficient adult mice, injection of hMSCs into the dentate gyrus of the hippocampus increased propagation, migration and neural differentiation of endogenous neural stem cells [30]. Also, injection of hMSCs into the dentate gyrus of immunocompetent mice one day after transient global ischemia improved neurological deficits and decreased neuronal death by the modulating immune and inflammatory responses of the mouse brain [31]. In this study, we tested the hypothesis that implantation of hMSCs into the striatum may have beneficial effects in a transgenic mouse model for HD. The cells increased neurogenesis and decreased atrophy of the striatum.

Results

Grafted hMSCs Do Not Survive in the HD Striatum

Previous studies demonstrated that hMSCs implanted into the dentate gyrus disappeared over 5 to 7 days in both immunodeficient and immunocompetent mice [30,31]. To determine whether implantation of hMSCs would have a similar effect in a mouse model for HD, 100,000 hMSCs were implanted into the striatum of 8 wk old mice (HD N171-82Q) and the brains were examined after 1, 3, 5, 7, 15, and 30 days (n = 5–6). Microscopy for the GFP cells (Figure 1A) at 1 day post-implantation showed that the MSCs had engrafted. Most of these cells survived for at least 3 days post-implantation as cells which retained the spindle-
shaped morphology of MSCs (Figure 1A, inset images). By 5 to 7 days post-surgery, the size of the graft was smaller and fewer GFP-hMSCs were present. By 15 days post-implantation, only remnants of the GFP-hMSCs, and no intact cells, were found (Figure S1).

The total number of grafted GFP-hMSCs was also estimated using stereological counting (Figure 1B) on the same sections used for the immunostaining. Only intact hMSCs with typical spindle-shaped morphology were included in this analysis (Figure 1A, inset images). Of the 100,000 cells that were implanted in the striatum, only 15.1% survived the first 24 hours post-operatively (15,063±3,776 cells). There was no significant change at 3 days, with approximately 17.3% of the grafted cells remaining (17,293±3374 cells, p = 0.573). The number of surviving GFP-hMSCs was significantly reduced between 3–5 days post-transplantation, with only 4.5% of the cells remaining (4,401±1678 cells, p = 0.004) and about the same number at day 7 (Not Significant change). At 15 and 30 days post-implantation, intact GFP-hMSCs were not detected.

Grafting hMSCs into the HD Striatum Significantly Increases Endogenous Cell Proliferation

Previous observations indicated that grafting hMSCs into the hippocampus of healthy mice increased the proliferation of endogenous neural cells [30]. To determine whether hMSCs enhanced the proliferation of endogenous cells in an HD mouse model, the number of proliferating cells in the SVZ and striatum was assessed by injecting mice with BrdU every 12 hours for the first 7 days post-implantation, thus labeling all actively dividing cells during the first 7 days and slowly proliferating cells from days 7–30. Immunostaining indicated an increase in the number of BrdU+ cells in the hMSC-implanted hemisphere within the first day post-implantation when compared to the PBS-injected, contralateral hemisphere (Figure 2 A, E). At 3, 5, 7, and 15 days post-implantation there were still more BrdU+ cells in the hMSC-implanted hemisphere than in the contralateral, PBS-injected, control hemisphere (Figure 2 B–D compared to F–H and I compared to L). At 30 days post-implantation, there was a marked decrease in the number of BrdU+ cells in both hemispheres.
(Figure 2 J, M). As a control, dead hMSCs were implanted into the HD striatum. There was no increase in the number of BrdU^+ cells in the dead hMSC-implanted hemisphere compared to the contralateral PBS-injected hemisphere when analyzed by immunostaining at 7 days post-implantation (Figure 2 K, N). At 5 days post-implantation intact GFP-hMSCs can still be detected; however, confocal microscopy confirmed that these cells were not BrdU^+ (P-S).

The number of BrdU^+ cells in each hemisphere was also counted by stereology (Figure 2 O). In the hMSC-implanted hemisphere, there was a progressive increase in BrdU^+ cells from 5 to 15 days post-implantation. In the PBS-injected hemisphere, there was a small but not significant increase on day 7 in BrdU^+ cells. Most importantly, however, there were significantly more BrdU^+ cells in the hMSC-implanted hemisphere than the contralateral, PBS-injected hemisphere at days 5 (p = 0.0421), 7 (p = 0.0295), and 15 days (p < 0.001). By day 30, the number of BrdU^+ decreased markedly. Implantation of dead hMSCs had no effect on the number of BrdU^+ cells.

**BrdU^+ Cells Underwent Neurogenesis**

To determine whether the BrdU^+ cells differentiated, sections were co-labeled with markers of differentiation. At 7 days post-implantation, a small number of the BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images). By 30 days post-implantation, there was an apparent increase in BrdU^+ cells co-labeled with nestin (Figure 3 A, inset images).
nestin, but the cells appeared immature with very short processes or without processes (Figure 3 G, inset images). Also, at 7 days post-implantation, many BrdU+ cells co-labeled with NeuN (Figure 3 B, inset images). By 30 days post-implantation, BrdU+/NeuN+ cells were present but most of the BrdU+ cells still did not express NeuN (Figure 3 H, inset images). By 30 days post-implantation, some BrdU+ cells expressed II1 tubulin (Figure 3 I, inset images). Therefore, a subset of BrdU+ cells differentiated into neurons expressing NeuN and II1 tubulin within the first 30 days post-implantation. Also, confocal imaging of sections demonstrated that a fraction of the BrdU+ cells were present as astrocytes and even less as microglia/macrophages (Figure S2). These results suggest that after MSC implantation, BrdU+ cells have the ability to differentiate into neurons and neural progenitor cells within the first 7 days. However, neurogenesis did not appear to be the primary method for repair.

Grafting hMSCs Appeared to Recruit Pre-Existing Neuronal Cells to the Striatum of HD Mice

In addition to the BrdU+ cells that underwent neurogenesis into neuronal cells, there was also an increase in the number of BrdU negative cells that were nestin+, NeuN+, and III1 tubulin+ in the hMSC-implanted hemisphere compared to the PBS-injected hemisphere. At low magnification at 7 days post-implantation, there was a slight increase in the number of Nestin+ cells in the hMSC-implanted hemisphere when compared to the contralateral, PBS-injected hemisphere (Figure 3 A, D). By 30 days post-implantation, there was a considerable increase in the number of nestin+ cells in the hMSC-implanted striatum compared to the PBS-injected, contralateral hemisphere (Figure 3 G, J). There was also an increase in the overall number of neurons in the HD striatum after hMSC implantation compared to the PBS-injected control when assessed by NeuN staining at 7 (Figure 3 B, E) and 30 days (Figure 3 H, K). At 7 days post-implantation, there was a slight increase in III1 tubulin+ neurons (Figure 3 C, F). However, by 30 days, the relative number of III1 tubulin+ neurons in the MSC-implanted compared to the PBS-injected hemisphere had increased substantially (Figure 3 I, L). The results indicated either that some neural stem cells whose proliferation was stimulated by the hMSCs were not labeled by BrdU, the hMSCs recruited other progenitor cells the striatum in the HD mice, or hMSC implantation was neuroprotective and increased survival of pre-existing, endogenous striatal neurons. There was no apparent increase in astrocytes, microglia, and macrophages an observation consistent with the fact that the mice were immunosuppressed (Figure S3).

Grafting hMSCs in the HD Mouse Striatum Increased Neurotrophin Signaling

Assays for FGF-2 signaling indicated a substantial increase in the striatum at 7 days post-implantation when compared to the contralateral, PBS-injected hemisphere (Figure 4 A, E) and a further increase by 30 days post-implantation, especially surrounding the SVZ (Figure 4 I, M). CNTF was not increased at 7 days post-implantation (Figure 4B, F) but there was an increase in the striatum proximal to the SVZ 30 days post-implantation (Figure 4J, N). VEGF signaling was increased at 7 days post-hMSC implantation (Figure 4 C, G), and the increase persisted at 30 days in the areas surrounding the original location of the hMSC graft as well as in the SVZ, whereas no VEGF signaling was detected in the contralateral, PBS injected-hemisphere (Figure 4 K, O). NGF signaling was similarly increased in the striatum at 7 days after hMSC implantation (Figure 4D, H) but only slightly increased in the SVZ at 30 days (Figure 4L, P). Therefore, hMSC implantation primarily increased FGF-2 and VEGF signaling at 7 days post-implantation, with increased signaling of FGF-2, CNTF, and VEGF by 30 days post-implantation.

hMSC Decreased Atrophy of the Striatal Volume in HD Mice

To assess the effects of hMSCs on HD pathophysiology, hMSCs were implanted into one hemisphere of the striatum and PBS injected into the contralateral striatum in 2-month old HD mice. One month post-implantation, the hMSC-implanted striatum appeared larger than the contralateral, PBS-injected striatum (Figure 5 Ai–Av). This effect was most noticeable in the rostral striatum, close to the implantation site, but was studied throughout 300 μm of the striatum. There were no apparent differences in the contralateral hemispheres of the striatum in wildtype mice (Figure 5 Bi–Bv). Total striatal volume of each experimental and control hemisphere was quantified using stereology. The size of the striatum in wildtype mice was approximately 7×10^9 μm^3 and is significantly smaller in HD mice (approximately 3.3×10^9 μm^3). The MSC-implanted striatum was significantly larger than the contralateral, PBS-injected hemisphere (approximately 6.3×10^9 μm^3, Figure 5C) in 3-month old HD mice. The HD, PBS-injected hemisphere was significantly smaller that the wildtype control hemispheres, but the HD, MSC-implanted hemisphere was not. Therefore, MSC implantation significantly decreased atrophy of the striatum.

Discussion

This study tested the hypothesis that hMSCs are neurogenic in the striatum of HD mice by increasing the endogenous cell response. Previous reports demonstrated that hMSCs disappeared through necrosis or apoptosis in 5 to 15 days post-implantation into the dentate gyrus of the hippocampus [30] or wildtype adult mice [31], and rat MSCs disappeared at about the same rate after implantation into the striatum of adult rats [32]. A similar rate of disappearance was observed here after implantation of hMSCs into the striatum of adult HD mice. Therefore there was little opportunity for the cells to replace neuronal cells that are lost in HD. Instead, the cells stimulated proliferation and differentiation of endogenous neural cells as indicated by the BrdU labeling experiments. There was also an increase in III1 tubulin+ neurons in the striatum that were not BrdU-labeled, an observation suggesting either that there was recruitment of non-stem cells to become neurons, increased neuronal survival or pre-existing neurons, or some neural stem cells escaped labeling with BrdU. The increase in neurogenesis may in itself account for the decreased atrophy of the striatum in the treated HD mice compared to PBS injected controls (Figure 5). However, the hMSCs may also have exerted a neuroprotective effect as was observed in a mouse model for transient cerebral ischemia [31]. Both the increased neurogenesis and any neuroprotective effects were probably exerted through the increase in expression of FGF-2, CNTF, VEGF, and NGF. The increases in FGF-2, CNTF, and VEGF persisted for at least 30 days, long after no human cells were detectable. Therefore the transient presence of the hMSCs must have stimulated prolonged expression of neurotrophins by endogenous neural cells in the HD brain. Similar effects were observed after implantation of hMSCs into the dentate gyrus of wildtype, adult mice [30] and of adult mice following transient cerebral ischemia [31]. The results here are consistent with the observation that implantation of autologous bone marrow stromal cells produced improvement in neurological defects in HD mice,
experiments in which the fate of the cells was not investigated [33]. The present study is not focused on clinical improvement in treated HD mice but the neurological consequence of an hMSC graft in HD mice; however, a more rigorous study to determine the age of treatment and variability in the phenotype is critical.

The improvement in neurogenesis and striatal volume observed in the HD mice supports previous suggestions that therapy with hMSCs might be effective in CNS diseases such as HD. However, a number of further questions need to be resolved such as appropriate time of the therapy and optimal doses. For example, by simply advancing the surgery by 2 weeks to 6 week-old mice, MSC implantation no longer had a significant effect on striatal volume (data not shown). Administration of MSCs may or may not have long-term benefits, since the endogenous neural cells that undergo neurogenesis contain the mutated gene. However, the hMSCs might reset the time clock for development of the disease phenotype. Observations in models for stroke suggest that intravenous administration of MSCs or conditioned medium from MSCs can be effective [27,29]. Therefore repeated administration of either the cells or soluble factors produced by the cells may be feasible.

Conclusions
We have demonstrated that hMSCs affect endogenous cells in the striatum of HD mice by increasing cell proliferation, neuronal differentiation, and neuronal cell recruitment. Furthermore, implantation of hMSCs into the striatum of HD mice prevented striatal atrophy associated with HD. We have documented specific cellular changes in the HD striatum after hMSC implantation. hMSCs are isolated from the bone marrow and, therefore, are readily available for potential therapy or treatment. The results therefore suggested that neural implantation of hMSCs may be of benefit in HD but a number of parameters of dose, treatment schedule, and route of administration need to be optimized.
Cell Culture

Frozen vials of extensively characterized preparation of hMSCs from normal healthy donors were obtained from the Tulane Center for the Preparation and Distribution of Adult Stem Cells (http://www.som.tulane.edu/gene_therapy/distribute.shtml) under an IRB approved protocol and expanded as described previously [34]. In brief, vials of passage 1 hMSCs (about $1 \times 10^6$ cells) plated in 15 cm-diameter dishes in complete hMSC medium [α-MEM (GIBCO/BRL, Grand Island, NY); 20% pre-screened FBS (Atlanta Biologicals, Norcross, GA); 100 units/ml penicillin; 100 μg/mL streptomycin; and 2 mM L-glutamine (all from GIBCO/BRL)]. After 24 h, the viable adherent cells were washed with phosphate buffered saline (PBS, pH 7.2) and lifted with 0.25% trypsin/1 mM EDTA at 37°C for about 5 min [11] to recover viable cells. The cells were replated at 50 to 100 viable cells per cm² in hMSC medium, incubated with a change of medium every 3 or 4 days for 7 days until reaching about 70% confluent, and lifted with trypsin/EDTA. The epitope profile of cells was: Negative for hematopoietic markers (CD34, CD36, CD117 and CD45), and positive for CD29 (95%), CD44 (>93%), CD49c (99%), CD49f (>70%), CD59 (>99%), CD90 (>99%), CD105 (>99%) and CD166 (>99%). For transplantation, the cells were washed with PBS, centrifuged, and re-suspended in PBS at 25,000 cells/μL. To create Green Fluorescent Protein (GFP)-hMSCs, GFP lentivirus with an ubiquitin promoter [35] and polybrene were added to the culture media for 24 hours. At least 95% of the hMSCs were GFP⁺. GFP-hMSCs were re-plated post-surgery to confirm viability (Figure S4).

Stereotactic Surgery

All protocols involving animal care and handling were approved by Emory University’s Institutional Animal Care and Use Committee. N171-82Q (Jackson Laboratories, Bar Harbor, Maine) 8-week old mice were used for stereotactic surgery. They were immunosuppressed with 10 mg/kg cyclosporine A administration subcutaneously daily for 3 days prior to and 15 days after surgery. During Cyclosporine A treatment, mice were housed in sterile cages with sterile food and water. After pre-treatment with 0.04 mg/kg Atropine, they were anesthetized using 90.0 mg/mL ketamine and 9.1 mg/mL xylazine intramuscularly. The cranium was exposed and 4 μL of hMSC suspension in PBS (25,000 cells/μL) were infused at a rate of 0.5 μL/min at: +0.74 anterior/posterior, +1.5 medial/lateral, –2.5 dorsal/ventral relative to Bregma [36] into the right striatum. The dwell time was 5 min. The same volume of PBS was injected at the same rate in the left hemisphere. The wound was closed using Vetbond (Fisher Scientific, Inc.) and mice were administered 2.5 mg/kg Flunixin for recovery. For labeling of stem cells, 50 mg/kg bromodeoxyuridine (BrdU) neutralized in PBS with 0.007N NaOH was administered intraperitoneally at the time of surgery and at 12 hour intervals for 7 days.

Perfusion and Brain Tissue Processing

All protocols involving animal care and handling were approved by Emory University’s Institutional Animal Care and Use Committee. Mice were euthanized with avertin intraperitoneally, they were perfused through the left ventricle with ice-cold PBS followed by 4% paraformaldehyde. The brains removed and post-fixed in 4% paraformaldehyde overnight at 4°C, transferred to fresh 30% sucrose for 2 days and then frozen with 2-methylbutane. Sections were cut at 40–50 μm using a cryostat.

Figure 5. hMSC implantation decreased striatal atrophy in HD mice. Serial sections throughout the striatum were used for striatal volume analysis. Serial sections of the striatum are outlined in both HD mice receiving MSCs or PBS (Ai–Av) and control WT mice (Bi–Bv). There was an apparent increase in striatal volume after MSC implantation at 8 weeks compared to the contralateral, PBS injected hemisphere (Ai–Av). There was no apparent difference in the size of the striatum in the opposite hemispheres in WT mice (Bi–Bv). Scale bar = 1 mm. (C) Stereological analysis at 1 month post-implantation revealed a significant increase in striatal volume after hMSC implantation compared to PBS injection ($n=5–6$; *). The PBS-injected striatum was significantly smaller than both the MSC-implanted (a) and PBS WT control striatum (b). There was no difference between the two hemispheres in the WT control group. *, a, and b denote $p<0.05$ by ANOVA with Holms-Sidak post-hoc analysis. doi:10.1371/journal.pone.0009347.g005
**Immunohistochemistry**

Sections requiring detection of bromodeoxyuridine (BrdU) were first pretreated with 30 min in 2N Hydrochloric acid at 37°C followed by 15 min in borate buffer at room temperature. Sections were blocked using 5% serum in Triton-X 100. Incubation in the primary antibody was overnight at 4°C and in the secondary antibody was 1 hour at room temperature. Primary antibodies used include: BrdU (Abcam Inc, Cambridge, ME), Nestin (Chemicon International, Temecula, CA), neuronal nuclei (Chemicon International), βIII Tubulin (Chemicon International), glial fibrillary acidic protein (Chemicon International), Iba1 (Wako Pure Chemical Industries, Ltd, Richmond, VA), cilary neurotrophic factor (Santa Cruz Biotechnology, Inc.), fibroblast growth factor-2 (Santa Cruz Biotechnology, Inc.), nerve growth factor (Santa Cruz Biotechnology, Inc.), vascular endothelial growth factor (Chemicon International). Sections were mounted and coverslipped using Vectashield (Vector Laboratories, Inc, Burlingame, CA).

**Hematoxylin and Eosin Stain**

Sections were mounted and allowed to adhere to the slide overnight. Sections were incubated in Hematoxylin for 10 seconds and Eosin for 5 seconds. Sections were rehydrated with water and mounted with aqueous media.

**Microscopy**

All confocal images were taken using the Zeiss LSM 510 Meta Confocal and corresponding software.

**Stereology**

All sections to be used for stereology were sectioned at 50 μm and examined with a spinning disc confocal microscope. Stereo Investigator (MicroBrightfield Bioscience, Williston, VT) was used to count cells. Cells were counted in every sixth section. Each mouse for stereological assessment received an hMSC graft in the right hemisphere and a PBS injection in the left hemisphere. The total number of GFP+ was counted in every sixth section. No ratio was needed for the MSC graft volume estimates because the entire size of the graft was calculated. The entire striatum was outlined in every sixth section and randomly sampled to count for BrdU+ cells. For analysis, both the total number of BrdU+ cells in each hemisphere as well as the ratio of BrdU+ cells in the hMSC-implanted hemisphere to those in the PBS-injected hemisphere was calculated to reduce potential differences in data collection between subjects. To quantify the volume of the striatum, the area of the entire striatum was measured in every third section. Based on the area in each section, the number of sections, plus the thickness of each section, the total volume per hemisphere was calculated. The ratio of the volume in the MSC-implanted hemisphere to that of the PBS-injected hemisphere was also determined.

**Statistics**

A one-way ANOVA was used to determine if any of the groups were significantly different from the rest. A One-way RM ANOVA was also used to determine any differences within each group. The post-hoc test used was the Holm-Sidak test.

**Supporting Information**

**Figure S1** Confocal microscopy of GFP-hMSCs in vivo. High magnification confocal microscopy confirmed GFP-hMSCs did not divide in vivo. Intact GFP-hMSCs did not label (arrows) with BrdU at 1 day (A–D), 3 days (E–H), or 5 days (I–L). As hMSCs died, some GFP-hMSC remnants were phagocytosed by surrounding cells (arrowheads), including BrdU+ cells at 5 days (I–L), 7 days (M–P), 15 days (Q–T), and 30 days (U–X). Scale bar = 40 μm. Found at: doi:10.1371/journal.pone.0009347.s001 (8.78 MB TIF)

**Figure S2** BrdU+ cells underwent gliogenesis. (A) A few BrdU+ cells differentiated into astrocytes by 7 days post-implantation. (B) By 30 days, many BrdU+ cells underwent gliogenesis into mature astrocytes with long, elaborate processes. A few BrdU+ cells had differentiated into mature microglia/macrophages at 7 (C) and 30 days (D) post-implantation. Arrows = single-label examples. Arrowheads = co-label examples. Scale bar = 40 μm. Found at: doi:10.1371/journal.pone.0009347.s002 (10.19 MB TIF)

**Figure S3** Grafting hMSCs did not recruit pre-existing, endogenous glial cells. There was no difference in the number of GFAP+ astrocytes in the hMSC-implanted hemisphere (A, C) compared to the PBS-injected hemisphere (E, G) at 7 or 30 days post-implantation. There was also no difference in the number of Iba1+ microglia/macrophages at the site of the hMSC graft (B, D) compared to the PBS injection (F, H) at 7 or 30 days post-implantation. Scale bar = 50 μm. Found at: doi:10.1371/journal.pone.0009347.s003 (6.21 MB TIF)

**Figure S4** Morphology of hMSCs. hMSCs were plated at 100 cells/cm2 and grown to 70% confluency before harvesting for surgery (A). Cells were infected with GFP during their post-thaw, recovery phase. DIC images reveal the typical morphology of MSCs and GFP fluorescence microscopy showed almost 100% expression. Cells were re-plated immediately following surgery and still proved to be viable (B). Found at: doi:10.1371/journal.pone.0009347.s004 (3.41 MB TIF)

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**Author Contributions**

Conceived and designed the experiments: BRS DJP AWC. Performed the experiments: BRS AMC. Analyzed the data: BRS AWC. Contributed reagents/materials/analysis tools: BRS AMC. Wrote the paper: BRS DJP AWC.

**References**

1. Vassos E, Panaz M, Kladli A, Vassilopoulos D (2007) Higher levels of extraverted hostility detected in gene carriers at risk for Huntington’s disease. Biol Psychiatry 62: 1347–1352.
2. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, et al. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277: 1990–1993.
3. Myers RH, Growsdon JH, Bird ED, Feldman RG, Martin JB (1982) False-negative results with levodopa for early detection of Huntington’s disease. N Engl J Med 307: 561–562.
4. Roy RA, Hernandez J, Vogel-van der Vli M, van Ommeren GJ, Bruyn GW (1993) Duration of illness in Huntington’s disease is not related to age at onset. J Neurol Neurosurg Psychiatry 56: 98–100.
5. Joel D (2001) Open interconnected model of basal ganglia-thalamocortical circuitry and its relevance to the clinical syndrome of Huntington’s disease. Mov Disord 16: 407–423.
6. Curtis MA, Penney EB, Pearson AG, van Roon-Mom WM, Butterworth NJ, et al. (2003) Increased cell proliferation and neurogenesis in the adult human Huntington’s disease brain. Proc Natl Acad Sci U S A 100: 9025–9027.
7. Curtis MA, Penney EB, Pearson J, Dragonow M, Connor R, et al. (2005) The distribution of progenitor cells in the subependymal layer of the lateral ventricle in the normal and Huntington's disease human brain. Neuroscience 132: 777–788.

8. Curtis MA, Waltho JF, Synek B, Faull RL (2005) A histochemical and immunohistochemical analysis of the subependymal layer in the normal and Huntington's disease brain. J Chem Neuroanat 30: 53–66.

9. White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, et al. (1997) Huntington is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17: 404–410.

10. Tattersfield AS, Csonj RJ, Liu YW, Kells AP, Faull RL, et al. (2004) Neurogenesis in the striatum of the quinolinic acid lesion model of Huntington's disease. Neuroscience 127: 319–332.

11. Batista CM, Kippin TE, Willame-Morawek S, Shimabukuro MK, Akamatsu W, et al. (2006) A progressive and cell non-autonomous increase in striatal neural stem cells in the Huntington's disease R6/2 mouse. J Neurosci 26: 10452–10460.

12. Bjugstad KB, Zawada WM, Goodman S, Freed CR (2001) IGF-1 and bFGF protect striatum against excitotoxic damage. Exp Neurol 161: 259–272.

13. Palmer TD, Ray J, Gage FH (1995) FGF-2-responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain. Mol Cell Neurosci 6: 474–486.

14. Goulier C, Chalon S, Venier-Julienne MC, Bodard S, Benoit J, et al. (2000) Neuroprotection of nerve growth factor-loaded microspheres on the D2 dopaminergic receptor-positive-striatal neurones in quinolinic acid-brain-lesioned rat: a quantitative autoradiographic assessment with isodobenzamide. Neurosci Lett 288: 71–75.

15. Mesri P, Pean JM, Nerriere-Daguin V, Jollivet C, Brachet P, et al. (2000) Intracerebral implantation of NGF-releasing biodegradable microspheres protects striatum against excitotoxic damage. Exp Neurol 161: 259–272.

16. Emerich DF, Bruhn S, Chu Y, Kordower JH (1998) Cellular delivery of CNTF but not NT-4/5 prevents degeneration of striatal neurons in a rodent model of Huntington's disease. Cell Transplant 7: 213–223.

17. Dominici M, Le Blanc K, Mueller I, Saper-Cortenbach I, Marini F, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315–317.

18. Owen M, Friedenstein AJ (1988) Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found Symp 136: 42–60.

19. Caplan AI (2000) Mesenchymal stem cells and gene therapy. Clin Orthop Relat Res 367: 60–70.

20. Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276: 71–74.

21. Kopen GC, Prockop DJ, Phinney DG (1999) Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 96: 10711–10716.

22. Li Y, Chen J, Wang L, Lu M, Chopp M (2001) Treatment of stroke in rat with intracerebral administration of marrow stromal cells. Neurology 56: 1666–1672.

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

24. Ozawa M (2008) Systematic neuronal and muscle induction systems in bone marrow stromal cells: the potential for tissue reconstruction in neurodegenerative and muscle degenerative diseases. Med Mol Morphol 41: 14–19.

25. Haro NY, Malman DJ, Przyborski SA (2008) Mesenchymal stem cells as mediators of neural differentiation. CoarT Stem Cell Res Ther 3: 43–52.

26. Parr AM, Tator CH, Keating A (2007) Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant 40: 609–619.

27. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98: 1076–1084.

28. Holstetter CP, Schwarz EF, Hess D, Widenfalk J, El Manira A, et al. (2002) Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci U S A 99: 2199–2204.

29. Prockop DJ (2007) “Stemness” does not explain the repair of many tissues by mesenchymal stem/multipotent stromal cells (MSCs). Clin Pharmacol Ther 82: 241–243.

30. Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ (2005) Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci U S A 102: 18171–18176.

31. Ohtaki H, Ylostalo JH, Foraker JE, Robinson AP, Reger RL, et al. (2008) Stem/progenitor cells from bone marrow decrease neuronal death in global ischemia by modulation of inflammatory/immune responses. Proc Natl Acad Sci U S A 105: 14638–14643.

32. Coyne TM, Marcus AJ, Reynolds K, Black IB, Woodbury D (2007) Disparate host response and donor survival after the transplantation of mesenchymal or neural stem cells to the intact rodent brain. Transplantation 84: 1507–1516.

33. Lescaudron L, Unni D, Dunbar GL (2003) Autologous adult bone marrow stem cell transplantation in an animal model of Huntington's disease: behavioral and morphological outcomes. Int J Neurosci 113: 945–956.

34. Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ (2002) Intracarotid administration of marrow stromal cells: the potential for tissue reconstruction in neurodegenerative and muscle degenerative diseases. Med Mol Morphol 41: 14–19.

35. Zhang H, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang J, et al. (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453(7197): 921–924.

36. Franklin KBJ, Paxinos G (1997) The mouse brain in stereotaxic coordinates. San Diego: Academic Press.