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Mesenchymal Stem Cells as Cellular Vectors for Pediatric Neurological Disorders

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Abstract

Lysosomal storage diseases are a heterogeneous group of hereditary disorders characterized by a deficiency in lysosomal function. Although these disorders differ in their etiology and phenotype those that affect the nervous system generally manifest as a profound deterioration in neurologic function with age. Over the past several decades implementation of various treatment regimens including bone marrow and cord blood cell transplantation, enzyme replacement, and substrate reduction therapy have proved effective for managing some clinical manifestations of these diseases but their ability to ameliorate neurologic complications remains unclear. Consequently, there exists a need to develop alternative therapies that more effectively target the central nervous system. Recently, direct intracranial transplantation of tissue-specific stem and progenitor cells has been explored as a means to reconstitute metabolic deficiencies in the CNS. In this chapter we discuss the merits of bone marrow-derived mesenchymal stem cells (MSCs) for this purpose. Originally identified as progenitors of connective tissue cell lineages, recent findings have revealed several novel aspects of MSC biology that make them attractive as therapeutic agents in the CNS. We relate these advances in MSC biology to their utility as cellular vectors for treating neurologic sequelae associated with pediatric neurologic disorders.

Keywords

Mesenchymal stem cells; marrow stromal cells; lysosomal storage diseases; pediatric; central nervous system; cell therapy; migration; paracrine signaling

1. Introduction

Children suffer from a variety of hereditary disorders that manifest as a profound deterioration in neurological function with age. Among these disorders the lysosomal

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storage diseases (LSDs) are most common. LSDs represent over 40 genetic disorders that result from defects in lysosomal function, which leads to accumulation of glycosaminoglycans, glycoproteins, or sphingolipids in organs throughout the body. Although rare, collectively these diseases have an incidence of approximately 1 in 7000–8000 live births (Winchester et al., 2000). Depending upon the specific enzyme deficiency, distinct patterns of substrate accumulation occurs in organs resulting in a wide spectrum of clinical symptoms (Moses, 1990). Additionally, the time of onset to disease, which ranges from infancy to adulthood, as well as the degree of clinical involvement is influenced both by the specific inherited genetic mutation and the level of enzyme deficiency. In some LSDs abnormal accumulation of storage material occurs within cells of the brain and spinal cord, making neuro-degeneration a prominent feature of these disorders. Biochemical and pathological studies indicate that specific neural cell types possess different sensitivities to accumulated storage material, making distinct brain regions susceptible to disease. For example in Gaucher disease, which is caused by a deficiency of glucocerebrosidase, significant neuronal losses have been observed within the basal ganglia, nuclei of the midbrain, cerebellum, dentate nucleus, and hypothalamus (Espinas and Faris, 1969; Kaye et al., 1986). A recent analysis of autopsy samples from patients with all three forms of Gaucher disease indicated that neuronal loss predominated in type 2 and 3 patients but patients with type 1 disease presented with astrogliosis (Wong et al., 2004). In contrast, patients with Niemann-Pick type C (NPC) typically exhibit widespread neuronal atrophy at early stages of the disease but at later stages Purkinje neurons in the cerebellum become uniquely sensitive to degeneration (Walkley and Suzuki, 2004). Alternatively, Sandhoff and Tay-Sachs patients exhibit widespread apoptosis throughout the cerebral cortex, cerebellum, and brain stem that affects neurons, oligodendrocytes, astrocytes, Purkinje cells, micro-glia, and vascular pericytes (Huang et al., 1997).

A subset of LSDs, the leukodystrophies, manifest as a profound degeneration of white matter due to defects in myelin metabolism. For example, metachromatic leukodystrophy (MLD), one of the most common leukodystrophies, results from the inability to degrade sulfated glycolipids due to a deficiency of the lysosomal enzyme arylsulfatase A (Gieselmann, 2008). Some MLD patients have normal arylsulfatase A activity but lack an activator protein that is involved in sulfatide degradation (Kolter and Sandhoff, 2005). Both defects result in the intra-lysosomal accumulation of sulfatide compounds in neural and non-neural tissues. Pathological features include diffuse demyelination and metachromatic-staining granules in glial cells and macrophages. Central and peripheral myelination is abnormal with widespread loss of myelinated oligodendroglia in the CNS and segmental demyelination of peripheral nerves (Gieselmann, 2008; Gieselmann and Krageloh-Mann, 2010). Symptoms typically manifest during peak periods of myelin formation in post-natal development resulting in progressive loss of both motor and cognitive functions followed by death in approximately five years. However, the onset of the disease may be delayed until adolescence or adulthood depending on the degree of enzyme deficiency. Recently, several new causative mutations have been identified for this disorder (Cesani et al., 2009; Galla et al., 2013; Luzi et al., 2013).

In summary, many LSDs present with neurological sequelae but the cause and extent of neuro-degeneration are dictated by the nature of the accumulated storage material and its

relative toxicity to different cell types. Consequently, developing a single therapeutic approach to treat neurologic sequelae associated with LSDs is difficult since the therapy must reduce accumulated storage material in a variety of cell types localized within different anatomical regions of the brain. Presently, hematopoietic stem cell transplantation (HSCT) and umbilical cord blood transplantation (UCBT) attempt to achieve this result by providing the CNS with a continuous supply of micro-glial cells with normal lysosomal functions via the circulation. However, since the overall efficacy of these approaches remains uncertain, alternative treatment strategies designed to more efficiently target the CNS are under development.

2. Cell-Based Therapies for Lysosomal Storage Diseases

Over the past 25 years HSCT has been used to treat many types of sphingolipidosis and mucopolysaccharidosis (Krivit, 2002; Lund, 2013). This procedure provides hematopoietic cells from an unaffected donor to replace those of the host loaded with non-metabolized storage material. Moreover, enzyme produced by donor-derived cells or released during their turnover can accumulate in adjacent host cells or in plasma, reducing the amount of storage material both locally and systemically. HSCT has shown a measurable clinical benefit for the treatment of Hurler syndrome (Boelens et al., 2013; Guffon et al., 1998; Krivit et al., 1995; Peters et al., 1996; Sauer et al., 2009; Souillet et al., 2003), MLD (Biffi et al., 2008; Kidd et al., 1998a; Krageloh-Mann et al., 2013; Krivit et al., 1990; Malm et al., 1996), and Krabbe disease (Duffner et al., 2009; Gelinas et al., 2012; Krivit et al., 1998; McGraw et al., 2005; Pastores, 2009; Siddiqi et al., 2006). However, overall results in these patients are mixed as outcomes often exhibit marked between-subject variability. Moreover, some published case histories have reported no benefit to patients despite evidence of stable donor cell engraftment in bone marrow (Hoogerbrugge et al., 1995; Malm et al., 1996). Nevertheless, long-term follow up of Hurlers syndrome patients treated with HSCT indicated that the treatment does provide long-term, slow improvement in adaptive behaviors and psychomotor skills in children (Bjoraker et al., 2006; Lucke et al., 2007) albeit their continued development occurs at a slower than average rate and never accelerates to a normal range as compared to age-matched peers. As anticipated, children with good cognitive levels prior to HSCT and continued growth of cognition after HSCT also exhibited good adaptive functions.

Collectively, these studies reveal a clear benefit for early identification and treatment of patients with infantile forms of rapidly progressive neurodegenerative diseases. Consequently, UCB cells have gained prominence for the treatment of LSDs due to their rapid availability, enhanced engraftment potential, and reduced risk of graft versus host disease (GvHD) (Jaing, 2007; Mogul, 2000). UCBT has shown great benefit in delaying the onset of neurologic dysfunction when applied to storage disease patients who are asymptomatic at the time of the transplant (Martin et al., 2013; Prasad and Kurtzberg, 2010). Moreover, UCBT results in lower rates of graft failure, a higher incidence of full donor chimerism, normalization of serum enzyme levels, and a low rate of GvHD. Nevertheless, despite encouraging data regarding the effectiveness of both HSCT and UCBT (Boelens et al., 2013) both procedures suffer from several inherent complications associated with pre-conditioning, graft rejection, and development of acute and chronic GvHD that pose

significant risks to the patient (Grewal et al., 2003; Martin et al., 2006; Ozen et al., 2007; Prasad and Kurtzberg, 2010). Recent studies also indicate that long-term mortality rates remain significantly higher after HSCT in patients with inborn errors of metabolism (Eapen et al., 2012). Debate lingers regarding the role of the blood brain barrier in limiting access of proteinaceous or cellular therapeutics to the CNS, as well (Begley et al., 2008). Consequently, more research is needed to develop standardized criteria for patient selection, treatment regimens, post-treatment supportive care, and short and long-term outcome analyses such that the overall therapeutic benefit of HSCT and UCBT can be better evaluated (Abdelhalim et al., 2014; Escolar et al., 2006). Continued long-term analysis of functional outcomes in patients is particularly important owing to the fact that anecdotal reports have surfaced suggesting that many pre-symptomatic children treated with UCB transplants have developed motor and language deterioration (Duffner et al., 2009).

3. Intracranial Stem Cell Transplantation

Rodent, feline, canine, and non-human primate LSD models have been described and the neuropathology exhibited in these animal models recapitulates to a large degree that of human patients. Consequently, they have proved valuable for evaluating novel cell-based therapies. For example, Synder *et al*. (Snyder et al., 1995) and Meng *et al*. (Meng et al., 2003) first reported that neuro-progenitor cell lines engineered to express β-glucuronidase (GUSB) showed widespread engraftment when injected intra-cranially into a mouse model of mucopolysaccharidoses type VII (MPS VII), leading to a decrease in storage material throughout the brain. Similarly, Fukuhara *et al*. (Fukuhara et al., 2006) reported that intracranial injection of fetal neural stem cells into MPS II mice increased brain GUSB activity to ~ 6% of wild type levels by 3 weeks post-transplant resulting in reduced levels of storage material in the brain and improved non-spatial hippocampus-dependent memory. More recent studies have confirmed that neural progenitor administration can ameliorate disease in animal models of LSD (Givogri et al., 2008; Shihabuddin and Cheng, 2011; Strazza et al., 2009). Others have reported positive effects following intracranial transplantation of engineered amniotic epithelial cells (Kosuga et al., 2001) and neuro-progenitor cells (Lacorazza et al., 1996) in animal models of MPS VII or Tay-Sachs disease, respectively.

These and other studies prompted a phase I clinical trial to assess the safety of normal human central nervous system stem cells (HuCNS-SC) injected intra-cranially for treatment of Batten disease, a common form of a group of disorders referred to as ceroid lipofuscinosis, neuronal (CLN). At least ten genetically distinct forms of CLN exist, which have been associated with mutations in specific genes encoding soluble (CLN1, CLN2, CLN5, and CLN10) and trans-membrane (CLN3, CLN6, CLN7, and CLN8) proteins (Warrier et al., 2013). Defects in the activity of these proteins lead to excessive accumulation of lipo-pigments in cells of the brain, eyes, and other organs. For example, CLN1 results from a deficiency in the activity of palmitoyl protein thioesterase (PPT), a lysosomal enzyme that removes fatty acyl groups from cysteine residues on fatty acid modified proteins. CLN2 patients are deficient in tripeptidyl peptidase 1 (TTP1), a pepstatin-insensitive lysosomal peptidase that removes tri-peptides from the N-terminal of polypeptides (Bennett and Hofmann, 1999). All forms of CLN invariably prove fatal after a

prolonged period of disability. Moreover, neurological sequelae associated with Batten disease have proven refractory to bone marrow transplants. For example, Lonnqvist *et al*. (Lonnqvist et al., 2001) reported that three infants who received bone marrow transplants, two of whom were asymptomatic at the time of treatment, developed disease by 2 to 3 years of age despite the fact that PPT1 enzyme activity was normalized in peripheral leukocytes. Other studies have documented a complete lack of enzyme transfer between donor and patient lymphocytes *in vitro*, which may account in part for the ineffectiveness of bone marrow transplantation in patients (Lake et al., 1995). Results from human clinical trials are anticipated based on animal studies, which also demonstrated no efficacy of bone marrow transplantation in canine (Deeg et al., 1990) and sheep (Westlake et al., 1995) models of the disease.

The aforementioned clinical trial, conducted at the Oregon Health and Science University and sponsored by StemCells Inc., targeted both infantile and late infantile forms of CLN1 and CLN2. A total of six children were treated by direct intracranial injection of allogeneic HuCNS-SC. In January of 2008 a 9 year old girl who received bilateral stem cell injections succumb to the disease. Post-mortem examination of the patient’s brain did not reveal a tumor but there was marked brain atrophy and neurons contained high levels of undigested lipofuscin consistent with advance stages of disease progression. Nevertheless, long-term (one year) survival of the transplanted donor cells was evident in tissues samples taken at autopsy. The five remaining patients were enrolled in a four-year long-term observational study, with three of the five surviving to the end of the four-year study. Assessment of the patients' cognitive and neurological function revealed stable scores in some tests, but the clinical outcomes were generally consistent with the expected course of impairment associated with the disease. For example, magnetic resonance imaging scans of the brain showed progressive atrophy consistent with the patient's neuropsychological performance but quality-of-life measures remained stable across all three surviving patients. While no specific conclusions about impacting the disease course could be made in the open-label trial, no safety concerns attributed to the HuCNS-SC cell transplantation were noted (Selden et al., 2013). HuCNS-SC cell transplantation was also recently evaluated in a 1-year, open-label phase-1 study for the treatment of Pelizeaus-Merzbacher Disease (PMD), a fatal myelination disorder that afflicts male children (Gupta et al., 2012). Herein, allogeneic HuCNS-SCs were surgically implanted into the frontal lobe white matter in four male subjects with an early-onset severe form of PMD. Once again the HuCNS-SC transplantation was well tolerated and modest gains in neurological function were observed in three of the four subjects. Magnetic resonance imaging revealed increased myelination in the region of transplantation compared to control white matter regions remote to the transplant sites indicative of durable cell engraftment within the brain.

The use of HuCNS-SCs for the treatment of Batten’s disease and PMD is advantageous for several reasons. First, HuCNS-SCs retain the ability to differentiate into various neural cell types, and therefore may potentially replace host brain cells lost to disease. Second, transplantation of the cells into their native tissue environment typically yields reliable and widespread engraftment. However, these advantages are offset by the fact that all transplants are allogeneic in nature and therefore immuno-suppression is required to achieve durable,

long-term engraftment *in vivo*. Additionally, some Parkinson's patients who received grafts of fetal neural tissue developed adverse side effects in the form of various movement disorders due to aberrant integration of the donor neural cells into the host CNS circuitry (Freed et al., 2001). Therefore, benefits afforded by restoration of deficient enzymatic activity may be offset by morbidities associated with aberrant integration of donor-derived neurons into the established brain circuitry.

3.1. MSCs as Vectors for Neurological Diseases

MSCs derived from adult bone marrow have also demonstrated efficacy in treating various neurological disorders. Based on early studies demonstrating that they regulate hematopoiesis and differentiate into connective tissue lineages, MSC-based therapies were initially developed to treat osteogenesis imperfecta (Horwitz et al., 1999; Horwitz et al., 2002) and to speed recovery from bone marrow transplantation (Koc et al., 2000). More recently, MSCs have been shown to secrete a broad array of powerful paracrine-acting factors that promote tissue repair by providing trophic support, suppressing inflammation, and modulating immune cell function (Caplan and Dennis, 2006; Caplan and Correa, 2011; Nauta and Fibbe, 2007; Phinney, 2007) (Figure 1). Consequently, MSC-based therapies are now being evaluated in human clinical trials for treatment of a broad array of disorders including myocardial infarction (Hare et al., 2009), GvHD (Ball et al., 2013; Le Blanc et al., 2008), severe systemic lupus erythematosus (Wang et al., 2012), and complex peri-anal fistulas (Yong Lee et al., 2013).

Our laboratory was the first to demonstrate that bone marrow-derived MSCs injected directly into the CNS of newborn mice persistently engraft, migrate throughout a large volume of the brain, and at a low frequency acquire characteristics of neural cells (Kopen et al., 1999). Based on these and other related studies, MSCs were quickly exploited as cellular vectors to deliver deficient enzymes to the CNS in animal models of LSD. For example, intra-cranial injection of plastic adherent cells from murine bone marrow engineered to over express acid sphingomyelinase (ASM) into ASM-deficient mice were shown to exhibit widespread engraftment throughout the CNS, reducing brain levels of sphingomyelin, retard death of Purkinje cells, and prolonged animal survival (Jin et al., 2002). Combination therapy employing systemic administration of ASM-transduced bone marrow cells together with intra-cerebral MSC injection was also shown to restore ASM activity to near normal levels in most tissues including brain (Jin and Schuchman, 2003). This resulted in a marked reduction in sphingomyelin levels and preservation of greater than 80% of all Purkinje cells in the cerebellum. Sakurai *et al*. (Sakurai et al., 2004) further demonstrated that intracranial transplantation of MSCs engineered to express GUSB produced elevated enzyme levels in the olfactory bulb, striatum, and cerebral cortex, significantly reducing glycosaminoglycan levels and improving the cognitive function of MPS VIII mice. MSCs have also been shown to promote recovery in neurological function in experimental animal models of Parkinson's disease, Huntington's disease, Tourette syndrome, stroke, traumatic brain injury, and other disorders (Joyce et al., 2010; Phinney and Isakova, 2005; Teixeira et al., 2013). Collectively, these data demonstrate that MSCs are well-suited as vectors to treat neurologic sequelae associated with LSDs. Nevertheless, few if any studies have examined the molecular mechanisms that mediate MSC engraftment, survival, and migration in the CNS despite that

fact that a better understanding of these processes may facilitate improved treatment regimens to increase efficacy.

3.2. MSC Engraftment in the Rodent and Non-Human Primate Brain

To analyze the engraftment kinetics and map the anatomical distribution of MSCs transplanted directly into the CNS our laboratory designed PCR primers and a Taqman® probe that specifically target the mouse Y chromosome (McBride et al., 2003). We then used quantitative real-time PCR to analyze brain tissue of female mice injected with primary MSCs enriched from the bone marrow of male mice. These analyses revealed that MSC engraftment levels were significantly greater in neonatal vs. adult transplant recipients despite the fact that only a small percentage of injected cells survived *in vivo*. Analysis of coronal brain slices from transplant recipients further demonstrated that male DNA was distributed along the brain neuraxis in both neonatal and adult transplant recipients, with the highest engraftment levels localized near the site of injection (bregma) in forebrain as well as in the cerebellum. These results were validated by visualization of male MSCs in brain tissue sections via fluorescent in situ hybridization using a Y chromosome probe. This analysis further revealed that MSCs localized to the striatum, cerebral cortex, granular layers of the hippocampus and cerebellum, and white matter tracts in forebrain (McBride et al., 2003; Phinney et al., 2006).

As part of a pre-clinical safety trial, we also evaluated the engraftment of MSCs transplanted to the CNS of infant and young adult rhesus macaques (Isakova et al., 2006; Isakova et al., 2007). Herein, unmatched MSCs from a universal male donor were delivered via stereotactic injection into the caudate putamen of female recipients and at various times post-transplant affects on neural development, behavior, motor performance, and cognition were evaluated. After sacrifice at 3 or 6 months post-transplant, coronal brain slices from each transplant recipient were subdivided into specimens of comparable size and anatomical location, analyzed by RT-PCR using a Macaca sp. Y chromosome-specific probe (Isakova et al., 2006), and the data transposed onto a physical map of the macaque brain. These analyses revealed that overall MSC engraftment levels were on average 17.8-fold higher ($p<0.05$) in infant vs. young adult transplant recipients with a maximal observed difference of 180-fold. Furthermore, male DNA was found to be widely distributed throughout both brain hemispheres, the anatomical location of which overlapped significantly between transplant recipients in both age groups. Infant macaques that received MSCs exhibited the highest engraftment levels in tissue specimens encompassing the somato-sensory, primary motor, and auditory cortex, caudate putamen, striatum, and hippocampus. Moreover, engraftment levels averaged over all infant transplant recipients were shown to vary significantly between different anatomical locations in the brain and as a function of time post-transplant. Therefore, MSCs preferentially localized to specific but overlapping anatomical regions and redistributed along the brain neuraxis as a function of time post-transplantation (Figure 2). This result is in stark contrast to that reported for fibroblasts, which have demonstrated a very limited capacity to migrate beyond the site of injection in brain (Kawaja et al., 1991; Senut et al., 1995; Taylor and Wolfe, 1997). Transplant recipients were also subject to a battery of age- and species-appropriate tests, which failed to reveal any adverse affects of MSC transplantation on cognition, fine and course motor function, behavior, or

development. This outcome was obtained despite the fact that transplant recipients were monitored throughout a large proportion of their first year of life, during which social behavior, motor skills and cognitive abilities are rapidly developing. Collectively, these data indicate that MSCs disseminate non-randomly within the brain and generally exhibit low overall engraftment levels. However, significantly higher engraftment levels are achieved in infant vs. adult transplant recipients. This outcome is highly favorable with regard to therapy since LSDs with neurologic complications typically exhibit an early age of onset and early intervention is critical toward retarding or reversing disease progression.

4. Cell-Matrix Interactions Important for MSC Survival

An important aspect of the aforementioned studies is the striking similarity in engraftment kinetics and anatomical distribution of MSCs when transplanted to the CNS of mice and non-human primates. These results suggest that engraftment and cell migration may be regulated by a conserved process in the brain. MSCs are adherent cells and therefore must bind specific extracellular matrix proteins such as fibronectin, laminin, and collagens via receptor mediated processes to ensure survival (Meredith et al., 1993; Song et al., 2007; Song et al., 2010). Attachment to a given substrate invokes the formation of stress fibers, which activates specific cytoskeletal signaling complexes to repress apoptosis (Ingber, 2002). For example, activation of the PI3K/Akt pathway following integrin receptor engagement induces expression of the anti-apoptotic protein Bcl-2 thereby enhancing cell survival (Matter and Ruoslahti, 2001). Integrin-mediated attachment to fibronectin in cooperation with growth factors also stimulates the mitogen activated protein kinase cascade, which regulates key intracellular enzymes and transcription factors that promote cell growth (Miyamoto et al., 1995; Miyamoto et al., 1996; Renshaw et al., 1997; Short et al., 1998). These effects are exemplified by the fact that over expression of the fibronectin receptor ($\alpha5\beta1$ integrin) in cells leads to decreased cell proliferation, but this inhibition is overcome by attachment to fibronectin (Varner et al., 1995). In bone marrow, integrins and their receptors are known to play important roles in regulating tissue homeostasis. For example, integrins $\alpha4\beta1$ (VLA-4) and $\alpha5\beta1$ (VLA-5) make up the main adhesion receptors used by hematopoietic stem/progenitor cells to adhere to stroma (Teixido et al., 1992). These integrins bind to different regions of fibronectin (FN), VLA-4 and VCAM-1, which is expressed by marrow stromal cells. In addition to regulating cell migration, blocking antibodies to VLA-4 have also been shown to disrupt lymphopoiesis, myelopoiesis, and erythropoiesis *in vitro* as well as affect cell cycle progression in hematopoietic stem cells (Coulombel et al., 1997; Oostendorp and Dormer, 1997). Integrins and other cell adhesion molecules also play important roles in regulating osteoblast survival and differentiation (Bennett et al., 2001).

5. Neural Cell Adhesion Molecules and Cell Migration

Although MSCs express receptors for extracellular matrix proteins common to connective tissues including fibronectin, osteopontin, laminin, and collagens these proteins are not abundantly expressed within the CNS. Laminin-1, for example, is expressed during CNS development but exists predominantly in vessel basement membranes and in reactive glia in the adult brain (Hagg et al., 1989; Zhou, 1990). Laminin α-2 immuno-reactivity is evident in

dendrites and dendritic spines in selected areas of the adult brain, predominately in the hippocampus and other limbic structures, which suggests a role in synaptic function and plasticity (Tian et al., 1997). Similarly, fibronectin is expressed mainly in association with blood vessels (Milner and Campbell, 2002) but is also up-regulated in glial cells in response to seizures (Hoffman et al., 1998) and focal brain injury (Tate et al., 2007). Limited expression of these matrix proteins in the brain may account for the poor survival of MSCs following direct intracranial injection.

In contrast, various neural cell adhesion molecules, such as L1, N-CAM, and cadherin 2 (CDH2) are expressed in many regions of the mouse (Bartsch et al., 1989; Miragall and Dermietzel, 1992), rat (Wagner et al., 1992), and human brain (Navratil et al., 1997) during development and in adulthood. These adhesion molecules play important roles in structural development and cell migration. In the latter case, the polysialylated neural cell-adhesion molecule (PSA-NCAM) has been shown to be essential for migration of neuroblasts from the sub ventricular zone to the olfactory bulb (Ono et al., 1994). Mice lacking NCAM exhibit a dramatic reduction in the size of the olfactory bulb due to accumulation of neural precursors along the rostral migratory stream (RMS) (Cremer et al., 1994). More recent studies indicate that NCAM functions as an alternative signaling receptor for glial-derived neurotrophic factor, which is produced in the OB, distributed along the RMS, and functions as a chemo-attractant for migrating neuroblasts (Paratcha et al., 2006). Similarly, CDH2 has been shown to regulate migration of precerebellar neurons in the developing hindbrain (Taniguchi et al., 2006) and post-mitotic neuroblasts in the subgranular zone of the dentate granular cell layer (Seki et al., 2007). Conditional knockout of CDH2 in mice also results in nearly complete randomization of intra-cortical structures, indicating that this adhesion molecule plays an important role in sorting of cells between boundary layers in the CNS during development (Kadowaki et al., 2007).

5.1. Tangential Migration of Interneurons

Alternatively, a large number of interneurons migrate tangentially throughout the brain in response to guidance cues that function over long distances. These guidance cues include the netrin, semaphorin, and slit family of proteins. Briefly, netrins are adhesion molecules with similarity to laminin that bind to deleted in colon cancer (DCC), neogenin 1 (NEO1) or Unc5H family members (de Castro, 2003). Netrins also bind extracellular matrix components via a basic domain at their carboxy terminus, which modifies their ability to diffuse in the brain. The ability of netrins to repel or attract neurons (or axons) is dependent upon specific receptor/ligand interactions. For example, neurons that express DCC or NEO1 are attracted to netrins while those that express Unc5H family members are repelled by them. Netrins have been reported to attract tangentially migrating neurons that will form the inferior oliva and repel migrating hypothalamic neurons and cerebellar granule cells (Causeret et al., 2002; Marin and Rubenstein, 2001; Marin, 2013). Other studies have indicated that netrins may also regulate the migration of glial cells (Tsai and Miller, 2002).

Semaphorins constitute a large family (~30 members) of secreted or trans-membrane proteins. The class III family of semaphorins binds to members of the neuropilin (NRP) family of receptors, NRP1 and NRP2, which are expressed in overlapping but distinct

populations of neurons in the CNS (Kolodkin et al., 1997). Differences in the affinity of neuropilins for distinct semaphorins play a role in sorting of migrating interneurons in the brain. For example, Marin et al. (Marin et al., 2001) demonstrated that migrating cortical interneurons avoid entering the striatum because of a chemo-repulsive signal composed of semaphorin 3A and 3F secreted by striatal cells. Interneurons expressing neuropilins are directed to the cortex while those that do not are directed to the striatum. As anticipated, loss of NRP1 or NRP2 function results in an increased number of interneurons invading the striatum with concomitant alterations in the number and distribution of interneurons in the cortex.

Slit proteins also regulate interneuron migration during development. Three human homologs of the Drosophila Slit gene have been identified (Slit1–3) and these are secreted proteins that contain conserved protein-protein interaction domains including leucine-rich repeats and epidermal growth factor-like motifs (Itoh et al., 1998). Slit proteins bind to the roundabout homology (ROBO) family of receptors, which are members of the neural cell adhesion molecule family (Kidd et al., 1998b). Four ROBO family members have been identified in vertebrates to date (Huminiecki et al., 2002; Kidd et al., 1998b; Yuan et al., 1999) and these proteins have been shown to mediate homophilic as well as heterophilic adhesions (Hivert et al., 2002). During development, Slit expression is maintained in the ventricular zone of the lateral germinal eminence, where it functions to repel interneurons from the subventricular zone and direct them toward the cortex (Zhu et al., 1999). ROBO1 and ROBO2 show complimentary patterns of expression with respect to Slit, consistent with the fact that these molecules function to direct cortical interneurons along their appropriate tangential routes (Andrews et al., 2007). Interestingly, loss of ROBO1 function has also been reported to result in an increase in the number of interneurons entering the striatum during development (Andrews et al., 2006), suggesting that both ROB-Slit and NRP-Sema interactions play central roles in interneuron migration during development. A number of these mechanisms important for neural cell migration persist in the adult brain, albeit in a greatly diminished capacity (Ghashghaei et al., 2007; Goldman and Luskin, 1998). For example, Slit proteins have been shown to repel neuronal precursors migrating from the anterior subventricular zone to the olfactory bulb (Ghashghaei et al., 2007; Wu et al., 1999) and inhibit migration of inferior olivary neurons by silencing the attractive effects of netrin 1 (Causeret et al., 2002).

5.2. MSCs Express Neural Cell Adhesion Molecules and Guidance Receptors

We previously reported that MSCs express many of the aforementioned adhesion and receptor proteins important for cell migration in the CNS. Specifically, interrogation of the MSC transcriptome revealed expressed mRNAs encoding the neural adhesion molecules ninjurin 1 and CDH2 and the receptors NEO1, NRP1, NRP2, ROBO1 and ROBO4 (Phinney et al., 2006). The expression of these proteins was found to be conserved in mouse, non-human primate, and human MSCs. Moreover, immuno-fluorescent staining and FACS analysis revealed that expression of each protein was restricted to specific subpopulations of MSCs. Functional studies *in vitro* further revealed that binding of MSCs to CDH2 or netrin 1 (NTRN1) could be saturated in a dose-dependent manner. Moreover, binding to CDH2 or NTRN1 was inhibited to greater than 95% by pre-incubating cells with soluble CDH2 or a

neutralizing antibody against NEO1, respectively. Therefore, CDH2 and NTRN1 function as homo- and heterophilic adhesion molecules, respectively, in MSCs. MSC migration was also shown to be significantly stimulated by exposure of cells to semaphorin 3A, repulsive guidance molecule A and NTRN1. This response could also be inhibited by pre-treating MSCs with neutralizing antibodies to the NEO1 and NRP1 receptors, demonstrating specificity of these ligand/receptor interactions.

Many of the adhesion molecules and guidance receptors we've identified within MSC subpopulations have specified functions in bone and marrow. For example, CDH2 is known to be expressed by osteoblasts and to regulate their function by modulating cell-to-cell adhesion (Cheng et al., 2000). Similarly, NRP2, which also binds to specific isoforms of VEGF (Neufeld et al., 2002), is expressed in osteosarcomas and promotes increased tumor vascularity resulting in a poor prognosis for survival (Handa et al., 2000). Additionally, ROBO1 expressed on leukocytes has been shown to regulate chemotaxis in response to secreted Slit protein (Wu et al., 2001). This protein is also expressed during development in muscle and cartilage and during mouse limb morphogenesis (Vargesson et al., 2001). Most recently, Slit2 was reported to be expressed on MSC-like populations within the bone marrow niche (Smith-Berdan et al., 2012). Expression of NEO1 has not been described in MSCs or stromal cells to our knowledge but this protein is known to have a broad tissue distribution (Meyerhardt et al., 1997). Consequently, while these proteins likely contribute to the function of MSCs in marrow, they may fortuitously impart MSCs with the capacity to engraft and migrate within the CNS. This hypothesis is consistent with the observation that MSCs transplanted to the CNS localize to anatomical regions that express the aforementioned adhesion molecules and guidance cues/receptors, such as the striatum, cerebral cortex, hippocampus and cerebellum (Goldman and Luskin, 1998; Marillat et al., 2002; Watakabe et al., 2006). Continued analysis of the role played by these proteins is anticipated to reveal novel insight into the mechanisms regulating MSC engraftment and migration in brain, thereby aiding in the development of improved cellular vectors that can be targeted to specific brain regions.

6. Recent Advances in MSC Biology and Consequence for Neurodegenerative Disorders

In recent years the field of MSC research has undergone a major paradigm shift. Initially, MSCs were thought to promote tissue regeneration via direct cell replacement, which reflected their capacity to differentiate into a variety of mesenchymal cell types. Over the past decade, accumulating evidence indicates that MSCs achieve a therapeutic effect largely via drug-like action by secreting a diverse array of paracrine acting factors. The later include proteins that exhibit anti-apoptotic, angiogenic, anti-inflammatory, neuro-regulatory, immunomodulatory and trophic activity.

6.1. Anti-Inflammatory and Immuno-Modulatory Activity

Recently, a growing number of laboratories have documented that MSCs possess immuno-modulatory properties important in regulating immune cell activity. Several recent reviews have been published that cover this topic (English, 2013; Le Blanc and Mougiakakos, 2012;

Tolar et al., 2010). MSCs have also been shown to ameliorate injury-induced lung inflammation by suppressing expression of pro-inflammatory cytokines *in vivo* (Gupta et al., 2007; Ortiz et al., 2003; Ortiz et al., 2007). The anti-inflammatory and immuno-modulatory affects of MSCs may be advantageous in the treatment of LSDs since inflammation is a recognized aspect of disease that likely contributes to pathogenesis. For example, progressive, widespread glial cell activation has been reported in the brains of pre-symptomatic sheep afflicted with CLN (Oswald et al., 2005) and in a mouse model of this disease (Pontikis et al., 2004). Inflammation within the cerebral cortex is also evident by 1 month of age in mice afflicted with MPS I and IIB (Ohmi et al., 2003) and elevated levels of pro-inflammatory cytokines have also been detected in the brains of fetal mice afflicted with Gaucher disease (Hong et al., 2006). Progressive CNS inflammation also coincides with the onset of clinical symptoms in a mouse model of Tay-Sachs disease (Jeyakumar et al., 2003). In most cases, microglia activation is thought to occur in response to aberrant neural cell function or as part of a wider stress response in the brain and typically precedes neuronal cell loss. Several studies suggest that a vigorous inflammatory response contributes to disease pathogenesis. For example, bone marrow transplantation has been shown to suppress microglial activation and inhibit neuronal cell death in the absence of any detectable increase in neuronal GM2 ganglioside storage in a mouse model of Sandhoff disease (Wada et al., 2000). Treatment of Sandhoff mice with non-steroidal anti-inflammatory drugs has also been shown to result in a significant increase in life-span (Jeyakumar et al., 2004). In addition, animals lacking expression of the leukocyte chemokine MIP-1 alpha exhibit substantial decreases in the number of infiltrating macrophages into the CNS together with improved neurologic status and longer lifespan (Wu and Proia, 2004). Consistent with these findings, inflammation has also been reported as a prominent feature in other neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Das and Basu, 2008).

Consistent with their role in immune-modulation, injection of unmodified MSCs into the cerebellum was shown to markedly reduce the extent of microglial and astrocyte activation and reduce levels of macrophage colony stimulating factor, a microglial activator, in a mouse model of NP-C disease (Bae et al., 2005). Interestingly, neural stem cells have also been shown to inhibit inflammation when transplanted to the brains of Sandhoff mice (Lee et al., 2007). MSCs also exhibit neuro-protective effects through anti-inflammatory action in models of Parkinson's disease (Chao et al., 2009), Alzheimer's disease (Lee et al., 2012) and traumatic brain injury (Zhang et al., 2013). Therefore, stem cell transplants may have the added benefit of reducing inflammation associated with LSDs.

6.2. Paracrine Signaling

Genomics-based studies including work from our own lab have shown that MSCs secrete various neurotrophins and other proteins that promote neural cell survival and neurite outgrowth under stressful conditions and following injury *in vivo* (Crigler et al., 2006; Joyce et al., 2010; Pisati et al., 2007; Qu et al., 2007). This capacity likely is related to the fact that bone and marrow are innervated by nervous tissue and different stromal subtypes respond to sympathetic efferent input to directly modulate hematopoiesis (Phinney, 2002). Indeed, several studies indicate that paracrine signaling contributes to the therapeutic effect of MSC-

based therapies for the treatment of storage disease. For example, a recent study demonstrated that MSCs transplanted into the hippocampus of NP-C mice at an early stage of disease progression resulted in recovery of motor function due to enhanced neuronal cell survival and proliferation (Seo et al., 2011). Herein, MSC administration suppressed cellular apoptosis, resulted in increased PI3K/AKT and JAK2/STAT3 signaling, and promoted restoration of neurotransmitter homeostasis within the brain. A related study reported that embryonic NSCs from NP-C mice, which exhibited impaired self-renewal and decreased rates of neuronal differentiation, recovered these activities to a large extent when co-cultured with marrow-derived MSCs (Lee et al., 2013). Moreover, intra-cerebral transplantation of MSCs to NP-C mice augmented proliferation and neuronal differentiation of NSCs within the subventricular zone via release of chemokine (C-C motif) ligand 2 (CCL2). Other studies have shown that co-culture of immortalized marrow-derived MSCs with Schwann cells derived from Twitcher mice induced proliferation and neurite outgrowth grown in the presence of psychosine, the toxic substrate that accumulates in this disease and that the neuritogenic effect of MSCs was ameliorated by addition of neutralizing anti-BDNF antibodies (Miranda et al., 2011).

Consistent with these studies, several reports have shown that the bone marrow microenvironment is altered in patients with storage disease, affecting both hematopoiesis and MSC function. For example, a prospective analysis of MSCs from 10 patients with type 1 Gaucher disease (GD) revealed defects in cell growth, cell cycle abnormalities, and decreased capacities to differentiate into osteoblasts (Lecourt et al., 2013). GD-MSCs also secreted soluble factors that stimulated osteoclasts resorbing activities and exhibited a reduced capacity to support hematopoiesis in vitro. A separate study found that MSCs from Gaucher patients also exhibited a marked increase in COX-2, prostaglandin E2, interleukin-8, and CCL2 production compared with normal controls and that treatment of normal MSCs with the glucocerebrosidase inhibitor conduritol B epoxide also induced expression of CCL2 (Campeau et al., 2009). These results suggest that the altered secretome displayed by GD-MSCs may contribute to skeletal and immune disease manifestations in these patients. Patients with Hurler's syndrome also exhibit skeletal defects and examination of MSCs from these patients revealed an increased capacity to support osteoclastogenesis as compared to MSCs from unaffected controls (Gatto et al., 2012). The later was correlated with up regulation of the RANKL/RANK/OPG pathway in Hurler MSCs. Therefore, paracrine activities that contribute to the therapeutic activity of MSCs may by dysregulated in LSD patients and contribute to disease pathophysiology.

6.3. Not all MSCs are Created Equal

Although initially isolated from bone marrow, MSCs have been identified in a variety of other tissues and organs. The ubiquitous distribution of MSCs in most tissues is attributed to their similarity to peri-vascular cells *in vivo*. This concept originated from studies demonstrating that bone marrow-derived MSCs express antigens common to endothelial cells and pericytes, such as STRO1 (Gronthos et al., 1994), CD146 and 3G5 (Shi and Gronthos, 2003) and conversely that post-capillary venule pericytes from bone marrow and peri-vascular cells in most blood vessels exhibit MSC-like characteristics (Brachvogel et al., 2005; Crisan et al., 2008; Shi and Gronthos, 2003). Several studies have shown that peri-

vascular cells, pericytes and fibroblasts from different tissues closely resemble the surface phenotype of MSCs, exhibit similar genome wide expression profiles, and share similar functional properties based on qualitative *in vitro* assays (Covas et al., 2008; Crisan et al., 2008). Close examination of these data, however, reveal clear differences in expressed levels of lineage restricted mRNAs between pericytes and MSCs (Covas et al., 2008) and functional differences between cell types can be readily discriminated using rigorous *in vivo* assays (da Silva Meirelles et al., 2006). For example, in one study ectopic transplantation of bone marrow-derived MSCs yielded hetero-topic bone tissue whereas dental pulp-derived MSCs produced dentin and pulp tissue (Batouli et al., 2003). Similarly, the capacity to generate bone and cartilage is weaker for placental and adipose-derived MSCs as compared to those from bone marrow, and the contribution to muscle fiber formation *in vivo* is greater with post-natal skeletal muscle pericytes than bone marrow-derived MSCs (Dellavalle et al., 2011). Therefore, while MSCs from different tissues share similarities in phenotypes and gene expression profiles, differences in function may be distinguished experimentally provided the assays are sufficiently rigorous. Consequently, not all MSCs are equivalent and their unique tissue-specific attributes must be carefully evaluated prior to use in clinical therapy.

MSCs also exhibit profound species-specific differences in their biological properties. Nevertheless, these differences are rarely discussed with respect to outcomes obtained from translational studies. Species-specific differences in activity are particularly relevant in LSD research as several well-established mouse models exist that are widely employed in pre-clinical studies. Two prominent differences that exist between human and rodent MSCs are sensitivity to atmospheric oxygen and immunogenicity. In the former case, human MSCs exhibit robust growth in culture and are amenable to large scale expansion (Digirolamo et al., 1999; Sotiropoulou et al., 2006). However, development of methods to procure and expand primary mouse MSCs has been significantly more challenging due to the overall poor growth of these cells *in vitro* (Baddoo et al., 2003). Consequently a growing number of laboratories have adopted purification schemes that select for rapidly proliferating subpopulations which emerge from plastic adherent marrow cultures following long-term culture expansion (Al-Khaldi et al., 2003; Li et al., 2008; Meirelles Lda and Nardi, 2003; Peister et al., 2004; Sun et al., 2003). These subpopulations typically survive in culture for over 50 passages *in vitro* and as such resemble immortalized cell lines. Indeed, cell immortalization occurs at a much higher frequency in rodent vs. human populations due to differences in checkpoint control mechanisms (Prowse and Greider, 1995; Wadhwa et al., 2004). For example, growth restrictive conditions have been shown to select for cells with inactivating mutations in p53, a protein mutated in the vast majority of immortalized rodent cell lines (Harvey and Levine, 1991; Sherr and DePinho, 2000). Consistent with these findings, we recently demonstrated that the poor growth of primary mouse MSCs is due to oxidative stress induced by exposure to atmospheric oxygen and that oxygen-induced growth inhibition is p53 dependent (Boregowda et al., 2012). Consequently, long-term exposure of mouse MSCs to atmospheric oxygen selects for clones with reduced or absent p53 function, which allows escape from oxygen-induced growth inhibition. Therefore, many studies claiming to use primary mouse MSCs in reality employ clonally selected immortalized cell lines. This is problematic for two reasons. First, clonally selected

populations do not recapitulate all of biological properties inherent to the parent population. Second, MSCs that lack a functional p53 protein are insensitive to oxidative stress. Therefore, these cells are anticipated to exhibit enhanced engraftment and survival in vivo as compared to primary cells, particularly when implanted into inflamed or diseased tissue. Therefore, outcomes from these studies require careful evaluation especially when extrapolating results to the human condition.

As noted above, a growing body of literature exists that MSCs possess immuno-suppressive activity (English, 2013; Le Blanc and Mougiakakos, 2012), which has spurred the use of allogeneic human MSCs in clinical therapies. However, studies conducted in experimental animals indicate that allogeneic MSCs trigger donor-specific cellular and humeral immune responses *in vivo*. For example, pre-clinical studies conducted in rodents (Camp et al., 2009; Eliopoulos et al., 2005; Rossignol et al., 2009; Sudres et al., 2006), swine (Poncelet et al., 2007), equine (Pigott et al., 2013) and non-human primates (Isakova et al., 2010; Isakova et al., 2014) demonstrate that allogeneic MSCs induce measurable anti-donor T and B cell mediated responses. Indeed, the detection of donor-specific antibodies in the serum of transplant recipients provides clear evidence of allo-antigen recognition by B cells. Species specific differences in MSC allo-reactivity may result from the fact that allograft responses in humans are typically evaluated using mixed lymphocyte reactions *in vitro*, which is not a reliable predictor of their suppressor activity in human patients (von Bahr et al., 2012). Recent studies indicate that immuno-modulatory activity of MSCs may be determined by a balance between inhibitory and stimulatory factors (Zhou et al., 2013). Therefore, experimental conditions particularly with regard to *in vitro* studies may yield disparate outcomes. Therefore, caution is needed when extrapolating results obtained with allogeneic MSCs in translational models to clinical therapy in LSD patients.

7. Summary

MSCs posses a number of unique biological attributes that make them well suited as cellular vectors to treat neurologic disorders. They show durable and widespread engraftment in the CNS, which may be mediated by expressed neural adhesion molecules and guidance receptor proteins. They also express anti-inflammatory and immuno-modulatory factors as well as a host of neural regulatory proteins. Nevertheless, continued research is necessary to better define these unique aspects of MSC biology, determine how tissue and species-specific differences in biology affect therapeutic potency, and to develop methodologies to exploit these attributes in ways that yield maximal benefit to patients.

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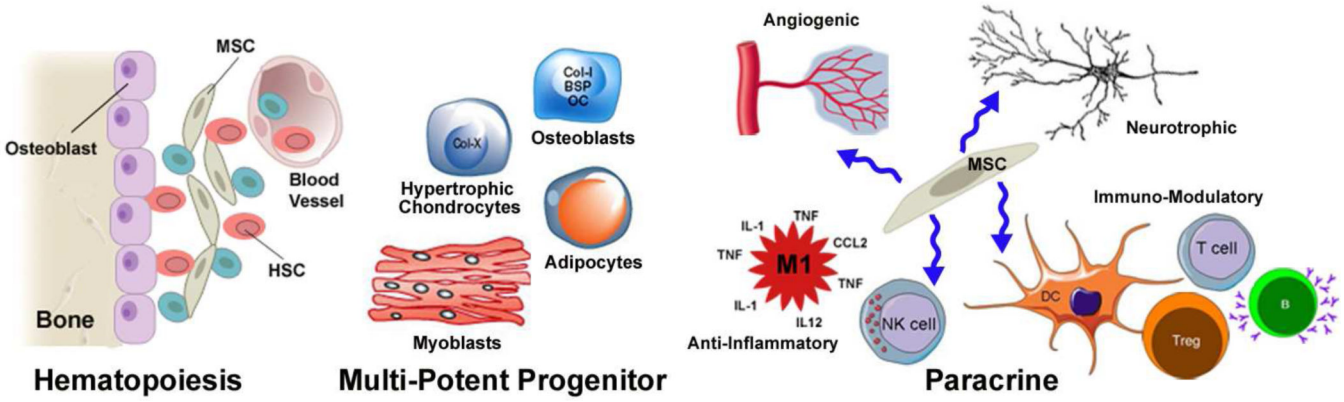

Figure 1. Biological functions of MSCs

MSCs contribute to the hematopoietic stem cell niche in bone marrow by promoting retention of quiescent HSCs within the endosteal niche. HSCs can be recruited from the niche to divide and yield progeny that enter the vasculature. MSCs also regulate tissue homeostasis by serving as a source of progenitor cells that differentiate into connective tissue lineages including adipocytes, chondrocytes, myoblasts, and osteoblasts. MSCs and their progeny also secrete a plethora of paracrine acting factors that exhibit angiogenic, neurotrophic, anti-inflammatory, and immuno-modulatory activity. In the latter case these factors act on cell types that comprise both the innate and adaptive immune system.

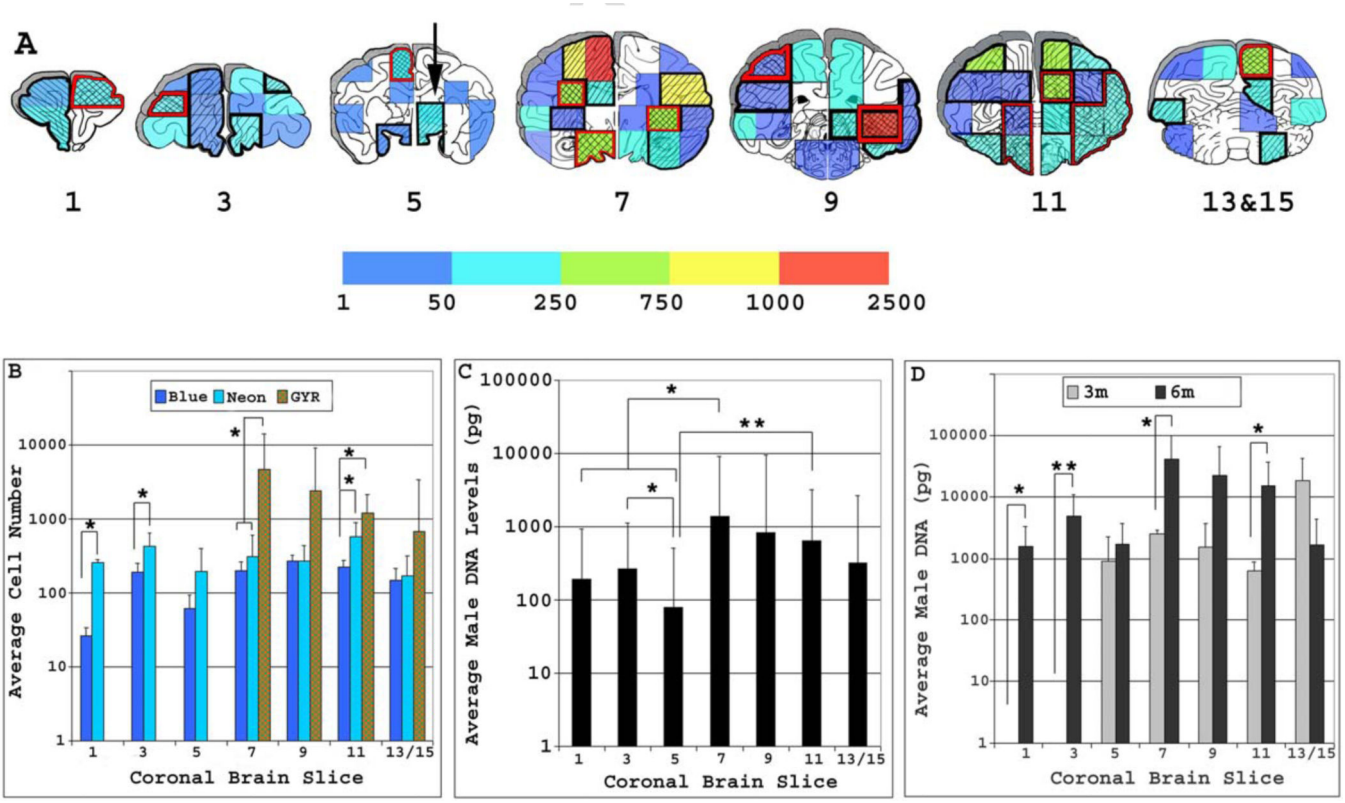

Figure 2. Anatomical distribution of MSCs engrafted in the infant macaque brain

A) Schematic showing the average number of male MSCs, which ranged between 1 and 2500 cells (colored bar) contained within equivalent brain specimens harvested from each transplant recipient. Brain specimens with overlapping engraftment between 2 (diagonal lines bordered in black) or more then 3 (hatched lines bordered in red) transplant recipients are denoted. Regions in white contained no detectable male DNA. B) Plotted is the average (mean ± SD) number of MSCs contained within different brain specimens from the same coronal slice. Significant differences in overall engraftment levels were evident between brain specimens containing between 1–50 (blue), 51–250 (neon) or 251–2500 (Green – Yellow-Red, hatched boxes) MSCs, *; $p<0.05$. C) Plotted is the average (mean ± SD) male DNA levels contained within each respective 3 mm coronal brain from all infant transplant recipients, *; $p<0.05$, **; $p<0.01$. D) Plotted is the average (mean ± SD) male DNA levels contained within each respective 3 mm coronal brain slice from all infant transplant recipients sacrificed at 3 or 6 months post-injection, *; $p<0.05$, **; $p<0.001$. Coronal brain slices are numbered 1–13 in a rostral-to-caudal orientation. Reprinted with permission from (Isakova et al., 2007).

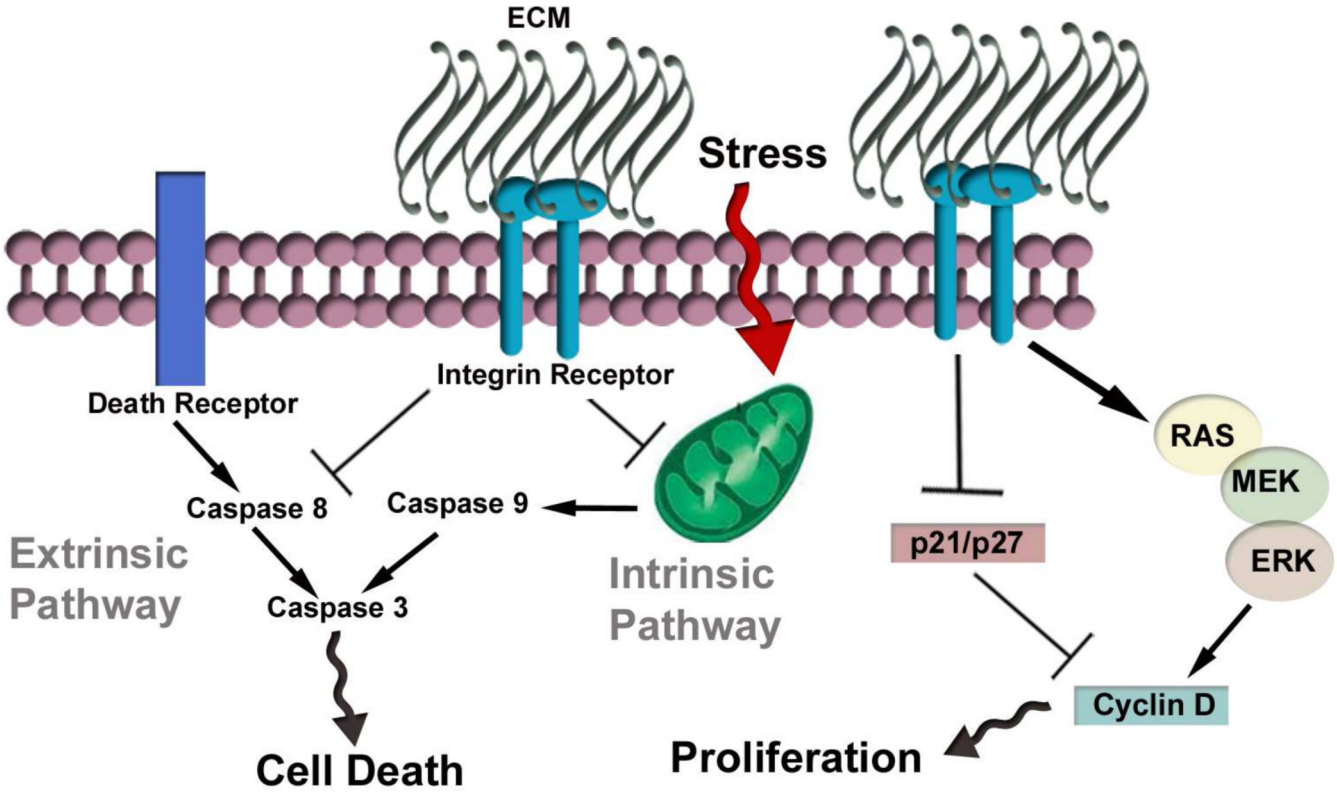

Figure 3. Integrin signaling regulates cell proliferation and survival

Growth factor signaling (not shown) and cell adhesion are required for transmitting signals to the Ras/Mek/Erk signaling pathway, which then stimulates cell proliferation via increasing cyclin D1 transcription and degrading the cyclin dependent kinase inhibitors p21 and p27. Signals from integrin receptors also repress the intrinsic and extrinsic cell death pathways. When both ECM and serum survival signals are absent activation of p53 dependent pathways induce cellular apoptosis.

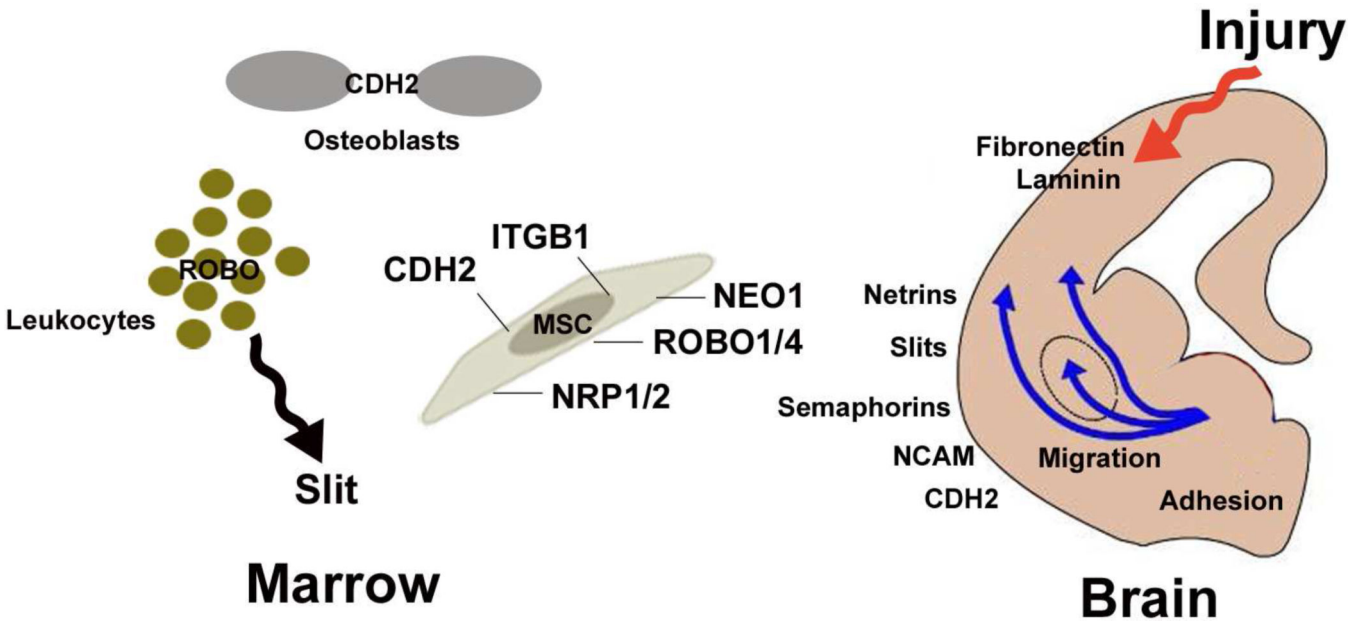

Figure 4. Proteins expressed by MSCs possess dual functionality in marrow and brain

MSCs express a variety of adhesion molecules and receptor proteins that perform specific functions in bone marrow. For example, MSCs express a broad array of integrin proteins that function as receptors for fibronectin, collagen, and laminin, which are widely expressed in marrow. MSCs also express CDH2, which is known to promote cell adhesion and regulate differentiation of osteoblasts. In addition, SLIT/ROBO interactions are known to regulate cheomtaxis of various leukocyte populations in marrow. Finally, NRP receptors regulate vessel formation by binding vascular endothelial growth factors and play a role in bone homeostasis by regulating osteoblast and osteoclast differentiation. Netrins, slits, semaphorins, CDH2, and NCAM also perform well described roles in neural cell survival, adhesion, and migration (see text) and several studies indicate that injury induces expression of fibronectin and laminin in the brain.