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MIGRATION AND FATE OF THERAPEUTIC STEM CELLS IN DIFFERENT BRAIN DISEASE MODELS

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Abstract

Stem cells have a number of properties, which make them excellent candidates for the treatment of various neurologic disorders, the most important of which being their ability to migrate to and differentiate predictably at sites of pathology in the brain. The disease-directed migration and well-characterized differentiation patterns of stem cells may eventually provide a powerful tool for the treatment of both localized and diffuse disease processes within the human brain. A thorough understanding of the molecular mechanisms governing their migratory properties and their choice between different differentiation programs is essential if these cells are to be used therapeutically in humans. This review focuses on summarizing the migration and differentiation of therapeutic neural and mesenchymal stem cells in different disease models in the brain and also discusses the promise of these cells to eventually treat various forms of neurologic disease.

Keywords

stem cell; pathotropic migration; differentiation; therapeutics

Stem cells are defined by their ability to continuously renew themselves by symmetric division and to give rise to more mature progenitors of multiple lineages through asymmetric division (Fig. 1). They can be isolated from both embryonic and adult human tissues, expanded and manipulated *in vitro*, and subsequently regrafted at which point they can differentiate into terminal cell types and integrate into living tissues (Gage, 2000; Barker and Wagner, 2003). These characteristics give stem cells enormous therapeutic potential, and because of this, stem cell-mediated therapies for a variety of diseases are actively being sought in both basic and clinical research. Many of these ongoing studies concern the use of stem cells for the treatment of neurologic disorders including but not limited to multiple sclerosis (Pluchino et al., 2009; Carbajal et al., 2010), brain tumors (Shah et al., 2008; Sasportas et al., 2009), stroke (Chu et al., 2003; Kim et al., 2004), and lysosomal storage diseases (Snyder et al., 1995). Of great importance to all of these efforts is the ability of therapeutic stem cells to successfully migrate to and function at targeted regions of pathology.

The efficient migration of stem cells toward regions of pathology in the brain followed by their successful integration, differentiation, and long-term survival at these sites is required for effective stem cell-mediated treatment of neurological disease. Recent studies in which therapeutic stem cells have been deployed *in vivo* into the brains of disease-bearing mice have shown that neural and mesenchymal stem cells (NSCs and MSCs) have these

capabilities. Specifically, NSCs and MSCs have been observed to travel to regions of neuropathology, differentiate predictably, and survive while stably expressing transgenes that they had been engineered to carry (Chu et al., 2003; Jeong et al., 2003; Ryu et al., 2004; Lee et al., 2007, 2009; Nagai et al., 2007; Shah et al., 2008; Pluchino et al., 2009; Sasportas et al., 2009; Carbajal et al., 2010). These findings, taken together with those of other studies concerning the migration and fate of therapeutic stem cells *in vivo*, demonstrate the great promise of this developing paradigm. However, before this potential can be realized in a clinical setting an in depth understanding of the physical and molecular mechanisms controlling the migratory properties and fates of therapeutic stem cells is necessary. In this review we summarize our current knowledge of therapeutic stem cell migration and fate in the mammalian brain. Furthermore, a brief overview of how these cells have been modified *in vitro*, imaged *in vivo*, and used to treat various animal models of neurologic disease will be discussed.

STEM CELL MIGRATION

A number of studies have shown that therapeutic stem cells transplanted into the mammalian brain preferentially migrate toward the regions of pathology. This was first demonstrated by Snyder and colleagues, who transplanted NSCs into a mouse model of mucopolysaccharidosis VII, a lysosomal storage disorder characterized by the fatal accumulation of glycosaminoglycans due to the absence of the enzyme β -glucuronidase. The NSCs, which were engineered to express β -glucuronidase and engrafted into the cerebral ventricles of newborn mice, were found to migrate extensively throughout the diseased brain and restore β -glucuronidase activity (Snyder et al., 1995). Stem cell pathotropism has also been observed toward ischemic (Veizovic et al., 2001; Chu et al., 2003; Jeong et al., 2003; Kim et al., 2004; Xu et al., 2007; Daadi et al., 2009), neoplastic (Shah et al., 2008; Lee et al., 2009; Frank et al., 2009; Sasportas et al., 2009), and demyelinating lesions (Pluchino et al., 2009; Carbajal et al., 2010). The migration of stem cells to pathologies such as these is highly efficient, so much so that murine NSCs injected into the tail vein (Aboody et al., 2000) or implanted into the contralateral hemisphere (Tang et al., 2003; Shah et al., 2005) of glioma-bearing mice are able to travel to and distribute throughout distant tumor masses (Fig. 2). This high efficiency and the pathotropism of transplanted stem cells are in large part due to cell signaling pathways that are activated during acute and chronic neurological dysfunction.

While stem cells have been shown to travel toward various neuropathologies *in vivo*, the molecular mechanisms governing this migration are at present incompletely understood. Studies of stem cell pathotropism have shown that a number of cytokine/receptor pairs are associated with the homing of stem/progenitor cells to diseased areas of the brain (Table 1), including stem cell factor (SCF)/c-Kit (Sun et al., 2004; Bantubungi et al., 2008), monocyte chemoattractant protein-1 (MCP-1)/chemokine receptor 2 (CCR2) (Widera et al., 2004; Magge et al., 2009), vascular endothelial growth factor/receptor (VEGF/VEGFR) (Zhao et al., 2008; Schmidt et al., 2009), hepatocyte growth factor (HGF)/c-Met (Heese et al., 2005; Garzotto et al., 2008), and stromal cell-derived factor 1 (SDF-1)/CXCR4 (Zhou et al., 2002; Imitola et al., 2004; Robin et al., 2006; Son et al., 2006; Carbajal et al., 2010). The first of these cytokines, SCF, is a membrane-bound protein that, when cleaved by proteases such as matrix metalloproteinase-9 (MMP-9), gives rise to a soluble peptide that binds to the tyrosine kinase receptor c-kit that is found on the surface of several stem/progenitor cell lines (Fazel et al., 2008; Huang et al., 2009; Souyima et al., 2009). The interaction of SCF with c-kit results in the activation of signaling pathways that have been implicated in the survival, proliferation, and chemotaxis of stem cells participating in hematopoiesis, gametogenesis, and melanogenesis (Ueda et al., 2002; Dentelli et al., 2007; Huang et al., 2009). SCF/c-kit signaling is also involved in the migration of exogenous NSCs toward

pathology within the brain, as demonstrated in mice subjected to “freeze” brain injury (Sun et al., 2004) and in a rat model of Huntington's disease (Bantubungi et al., 2008). In both of these studies, SCF was shown to be strongly upregulated by cells residing in and around lesioned areas. Furthermore, the blockade of stem cell c-kit was observed to significantly impair the migration of transplanted NSCs to the diseased striatum (Bantubungi et al., 2008). These studies provide convincing evidence that the interaction of SCF with its receptor c-kit plays an important role in the migration of stem cells toward regions of pathology within the brain.

MCP-1 is a chemotactic cytokine that promotes the migration of macrophages and monocytes through binding to CCR2 (Fuentes et al., 1995; Gunn et al., 1997). In addition to macrophages and monocytes, CCR2 is expressed by several other cell types, including memory T cells, regulatory T cells (T_{regs}), natural killer cells, and NSCs (Charo et al., 1994; Bartoli et al., 2001; Widera et al., 2004). Because of the expression of CCR2 by NSCs, it is not surprising that they migrate in the direction of an MCP-1 gradient, as shown by Magge and colleagues who demonstrated that NSCs travel toward MCP-1 infusion sites in the brains of healthy rats (Magge et al., 2009). MCP-1/CCR2 signaling has also been implicated in mediating the pathology-directed tropism of NSCs given that it has been found to induce the migration of stem cells toward inflammatory (Widera et al., 2004) and neoplastic (Magge et al., 2009) lesions within the mammalian brain. These studies reveal that, similar to SCF-1/c-kit, MCP-1/CCR2 signaling is a probable regulator of stem cell pathotropism.

VEGF is a protein mitogen that acts through the VEGFR to promote angiogenic endothelial cell proliferation and sprouting (Ferrara et al., 1992). VEGF is important in a number of physiological contexts, including development (Ferrara et al., 1996) and wound healing (Bates and Pritchard Jones, 2003). In addition to these roles in normal physiology, VEGF is also a major player in tumor angiogenesis and the hypoxic response, which is known to be activated in neuropathologies, such as ischemia (Bergeron et al., 1999) and cancer (Jensen et al., 2006). Hypoxia is known to promote NSC tropism *in vitro* (Xu et al., 2007) and *in vivo* (Zhao et al., 2008), mainly due to the upregulation of VEGF by hypoxic cells, which was observed to result in the increased expression of chemotactic factors Ang2 and GRO α (Schmidt et al., 2009). These two proteins, a growth factor and a chemokine respectively, promote the migration of NSCs toward regions of hypoxia within the brain and thus form the basis of the mechanism by which elevated levels of VEGF cause enhanced stem cell pathotropism.

Another cytokine/receptor pair involved in the migration of neural stem/progenitor cells in the brain is HGF, which acts through the cell surface c-Met receptor. HGF was initially characterized as a hepatocyte mitogen (Lundberg and Mollgard, 1979; Nakamura et al., 1984) and later studies established it as an important neurotrophic factor in the developing and adult central nervous system (Hobara et al., 2008; Nicoleau et al., 2009). Recent work has shown that the interaction of HGF with glioma cell c-Met results in the upregulation of chemokine receptor 4 (CXCR4) by tumor cells (Esencay et al., 2010), which is known to enhance their capacity for migration (Ehtesham et al., 2006; Stevenson et al., 2008). While the mechanism by which HGF/c-Met signaling mediates stem cell migration is at present unclear, it is likely that it involves the upregulation of CXCR4 as well (Son et al., 2006).

One of the best studied mediators of stem cell tropism is the G-protein coupled receptor CXCR4 and its only known ligand SDF-1. CXCR4 is among the most highly expressed chemokine receptors during both pre- and postnatal development (Jazin et al., 1997; Lavi et al., 1997). During development it is crucial for the proper formation of neural structures given that the deletion of either CXCR4 (Lu et al., 2002) or SDF-1 (Ara et al., 2003) has been shown to result in embryonic lethality. In the adult brain SDF-1 and CXCR4 continue

to be expressed at high levels, particularly in the dentate gyrus and subventricular zone where they mediate the tropism of endogenous NSCs (Tran et al., 2007). The SDF-1/CXCR4 axis therefore has a significant role in neural development and subsequent functioning, and recent studies have displayed that it is also important in directing the migration of therapeutic stem cells toward regions of pathology in the diseased brain. SDF-1/CXCR4 signaling has been reported to promote the migration of stem cells to diseased areas within the brain by a number of studies (Zhou et al., 2002; Imitola et al., 2004; Robin et al., 2006; Carbajal et al., 2010). Ischemic, neoplastic, and demyelinating lesions have been observed to induce the secretion of SDF-1 by reactive astrocytes, microglia, and endothelial cells (Zhou et al., 2002; Imitola et al., 2004; Thored et al., 2006; Carbajal et al., 2010), and our laboratory has recently collected data suggesting that CXCR4 is expressed by NSCs and MSCs at high levels (Duebgen et al., unpublished observation). The importance of the interaction between secreted SDF-1 and cell surface CXCR4 for stem cell migration has been displayed by experiments in which their activity has been inhibited. The blockade of both CXCR4 (Imitola et al., 2004) and SDF-1 (Carbajal et al., 2010) *in vivo* in diseased mice has been found to markedly reduce the migration of transplanted NSCs toward tumor foci and regions of demyelination, indicating that SDF-1/CXCR4 signaling is essential for effective pathotropism of therapeutic stem cells.

The SDF-1/CXCR4 system mediates the tropism of both endogenous and therapeutic stem cells by promoting chain migration, the principal mode of stem cell movement in the mature brain (Imitola et al., 2004). During early embryonic development, primitive neural structures are formed through the migration of NSCs along scaffolds created by specialized radial glia (Noctor et al., 2001). Because the adult brain lacks such scaffolding, endogenous stem cells in the dentate gyrus and subventricular zone must therefore rely on chain migration, a process characterized by α and β integrin-mediated homotypic interactions between migrating NSCs and tube-like structures formed by specialized astrocytes (Lois and Alvarez-Buylla, 1994; Jacques et al., 1998). The migration of therapeutic stem cells proceeds in a similar fashion to that of endogenous NSCs in that the transplanted cells employ chain migration to reach regions of neuropathology (Aboody et al., 2000; Tang et al., 2003; Shah et al., 2005). Through signaling pathways such as the SDF-1/CXCR4 axis and the chain migration that they mediate, therapeutic stem cells are able to undergo pathology-directed tropism within the brain, a phenomenon that has been observed in several animal models of neurologic disease.

A number of studies have shown that therapeutic stem cells transplanted into the brains of tumor-bearing mice migrate specifically toward areas of neoplastic growth. In addition to homing to main tumor burdens, engrafted NSCs and MSCs have been observed to track and efficiently clear malignant deposits located outside of the principal tumor mass (Aboody et al., 2000; Tang et al., 2003; Shah et al., 2005, 2008; Lee et al., 2009; Sasportas et al., 2009). This makes therapeutic stem cells particularly attractive candidates for the treatment of glioblastoma multiforme, a highly aggressive form of brain cancer characterized by the deposition of microscopic clusters of cancer cells outside of the main tumor burden that preclude surgical re-section and limit the effectiveness of conventionally delivered chemotherapies. In order to treat glioblastoma multiforme and other neurologic malignancies stem cells have been genetically modified to express transgenes encoding proapoptotic (Shah et al., 2004, 2005, 2008; Sasportas et al., 2009), antiangiogenic (van Eekelen et al., 2010), immunostimulatory (Yang et al., 2004; Dickson et al., 2007; Frank et al., 2009), and prodrug-converting (Aboody et al., 2000) protein products. Our laboratory has shown that stem cells manipulated in such a manner retain their migratory capabilities (Shah et al., 2008; Sasportas et al., 2009; Hingtgen et al., 2010; van Eekelen et al., 2010) and are therefore able to deliver therapeutics directly to tumors. We have displayed this in a number of contexts, most recently with NSCs engineered to produce a novel variant of the

antiangiogenic glycoprotein thrombospondin-1 (TSP-1). Transplantation of NSCs expressing TSP-1 into the brains of mice in the setting of malignant brain tumors resulted in markedly reduced tumor vessel density and the inhibition of tumor progression (van Eekelen et al., 2010). Our laboratory has also had great success with stem cells expressing a secretable form of tumor necrosis factor-related apoptosis-inducing ligand (S-TRAIL), a protein ligand that has been shown to preferentially target malignant cells while sparing normal brain tissue (Shah et al., 2005). We have found that both NSCs (Shah et al., 2008) and MSCs (Sasportas et al., 2009) engineered to secrete S-TRAIL are capable of tracking and efficiently clearing disseminated gliomas in mice. These results, taken together with those of the studies cited above, demonstrate the great promise of stem cell-mediated therapies for neoplastic lesions within the brain.

Stem cells engrafted into the brains of animal models of stroke and demyelinating disease have also been observed to migrate toward regions of ischemia and myelin depletion, respectively (Veizovic et al., 2001; Chu et al., 2003; Kurozumi et al., 2004). Many of the factors described above have been implicated in mediating this pathotropic migration, most notably SDF-1/CXCR4, which is known to have a role in the stem cell tropism that occurs in ischemia (Kelly et al., 2004). Recent work by Carbajal and colleagues has shown that the SDF-1/CXCR4 axis is also crucial to the pathotropic migration that takes place in demyelination. Through an elegant set of experiments involving SDF-1 and CXCR4 antagonism in the setting of myelin depletion, this group demonstrated that inhibition of CXCR4/SDF-1 signaling significantly reduced the migration and proliferation of engrafted stem cells as compared with unantagonized controls (Carbajal et al., 2010). Pathotropic migration, while essential for the effective treatment of these and other disorders via stem cells, is not sufficient to achieve a therapeutic outcome in ischemia and demyelinating disease. This is because, unlike in tumors, stem cells employed in these contexts must differentiate at sites of pathology in order to have an appreciable therapeutic effect.

STEM CELL DIFFERENTIATION

A number of studies investigating the fate of NSCs transplanted into the mammalian brain have shown that the extrinsic factors mainly drive their differentiation into either neurons or glia (Gage, 2000). The diseased adult brain appears to create an environment that stimulates endogenous and exogenous stem cells to differentiate into specific cell types. For example, both endogenous NSCs in the dentate gyrus and subventricular zone and those engrafted into the brain have been observed to preferentially undergo neurogenesis when subjected to ischemia (Zhang et al., 2001; Chu et al., 2003), thus replacing the cells most susceptible to ischemic insult. Similar differentiation to correct for the effects of pathology is evident in demyelinating lesions, where the majority of engrafted stem cells differentiate into oligodendrocytes resulting in the extensive remyelination of CNS axons (Pluchino et al., 2009; Carbajal et al., 2010). Contrary to what has been observed in ischemic and demyelinating lesions, therapeutic stem cells deployed into the brains of tumor-bearing mice remain in a quiescent and undifferentiated state (Aboody et al., 2000; Miletic et al., 2007; Shah et al., 2008; Sasportas et al., 2009; van Eekelen et al., 2010; Fig. 3). This failure of stem cells to differentiate when transplanted into the vicinity of neoplastic lesions has not been studied over long periods of time postengraftment; however, the results of the studies cited above strongly suggest that the tumor microenvironment simply lacks the factors necessary to promote stem cell differentiation into either neurons or glia.

A number of growth and transcription factors have been implicated as mediators of endogenous NSC differentiation. Research into the neurogenesis that takes place during early brain development has shown that multiple basic helix-loop-helix (bHLH) genes play a critical role in regulating the differentiation of NSCs into neurons (Ross et al., 2003). The

transcription factor products of these genes promote a neuronal fate by several mechanisms, including the activation of neuron-specific genes, the induction of exit from the cell cycle, and the inhibition of gliogenesis (Morrison, 2001; Shuurmans and Guillemot, 2002; Ross et al., 2003). There are two types of bHLH genes, the repressor type and the activator type. Repressor-type bHLH genes include the *Hes* genes which, in response to Notch signaling, antagonize activator-type bHLH genes resulting in the suppression of neuronal differentiation (Yoon and Gaiano, 2005). Activator-type bHLH genes include the *Mash* and *Ngn* families, which play an important role in promoting developmental neurogenesis. Activator-type and repressor-type bHLH genes regulate each other during brain development, allowing only subsets of cells to undergo differentiation and thus ensuring that others remain as NSCs. This precise regulation is essential for generation of complex brain structures of appropriate size, shape, and cellular arrangement.

During early brain development, astrocytes are produced in a second wave of differentiation, once most neurons have differentiated and migrated to their correct destinations within the developing brain (Gangemi et al., 2001). Effective astrocyte differentiation is dependent on Notch activation, which results in the downstream stimulation of *Hes1* and *Hes5*. As discussed above, these bHLH transcription factors suppress neurogenesis, and in doing so, they indirectly promote the astroglial differentiation program (Hirabayashi and Gotoh, 2005). Signal transducer and activator of transcription (STAT3) signaling, which is responsible for the expression of GFAP (a biomarker for astrocytes) has also been implicated in astrogenesis although in a more direct manner. When stimulated by cytokine growth factors, ciliary neurotrophic factor (CNTF), and leukemia inhibitory factor (LIF), STAT3 has been shown to trans-locate to the nucleus of neural stem/progenitor cells where it mediates the expression of genes known to promote astrocyte differentiation including but not limited to GFAP (Moon et al., 2002; Hirabayashi and Gotoh, 2005). The generation of astrocytes therefore requires both the inhibition of proneural transcription factors and the direct stimulation of proastrocytic genes by downstream effectors of STAT3.

Another condition that is of vital importance to the early generation of astrocytes is the inhibition of neural stem/progenitor cell differentiation into oligodendrocyte progenitors that are committed to an oligodendral lineage. This suppression is accomplished through the activity of the TGF- β family growth factor, bone morphogenetic protein (BMP). In addition to its induction of transcription factors that promote the expression of astrocyte-specific genes (Fukuda and Taga, 2006), BMP is able to indirectly facilitate the differentiation of astrocytes by inhibiting that of NSCs into oligodendrocyte progenitors via a mechanism that is at present unclear (Kasai et al., 2005; See et al., 2007). Although not resulting in increased oligodendrogenesis, the loss of BMP signaling in *bmpr* double knockout mice has been observed to significantly reduce the differentiation of NSCs into astrocytes both *in vitro* and *in vivo* (See et al., 2007). This indicates that while BMP activity is essential for the effective development of astrocytes, its downregulation is not sufficient for oligodendrocyte differentiation.

Oligodendrocytes are among the latest cells to differentiate in the nervous system, although oligodendrocyte precursor (OPL) cells are specified very early during development (Thomas et al., 2000). The process of oligodendral differentiation is mediated primarily by sonic hedgehog (Shh) signaling, which promotes the expression of the *Olig1* and *Olig2* genes. The bHLH transcription factor products of these genes, *Olig1* and *Olig2*, serve to commit NSCs to the oligodendrocyte developmental program (Lu et al., 2000; Zhou et al., 2002; Danesin et al., 2006). While Shh is the major promoter of developmental oligodendrogenesis, there is evidence that suggests that a Shh-independent pathway mediated by fibroblast growth factor 2 (FGF2) also plays a significant role. This was first reported by Chandran and colleagues, who found that treatment with FGF2 was able to induce the differentiation of NSCs into

OPL cells despite cyclopamine-mediated inhibition of Shh signaling (Chandran et al., 2003). Through both the Shh-dependent and -independent differentiation pathways neural stem/progenitor cells are thus committed to an oligodendral lineage; however, other factors are required for the further development and maturation of these cells.

After the specification of an oligodendral fate by either Shh or FGF2 signaling, other factors are required to mediate the further maturation and differentiation of OPL cells. One family of genes that has been implicated in this are the *Sox* genes of the E class, which includes *Sox8*, *Sox9*, and *Sox10*. Loss-of-function experiments have shown that mice without functional copies of either *Sox8* or *Sox9* generate markedly reduced numbers of astrocytes and oligodendrocytes (Cheung and Briscoe, 2003; Stolt et al., 2004a). This suggests that the protein products of these genes are involved in specifying a glial fate. *Sox10*, which is expressed exclusively in the oligodendrocyte lineage, is essential for the maturation of committed OPL cells. This was also established through loss-of-function studies, in which *Sox10* double knockout mice were found to have normal numbers of oligodendrocyte precursors that nevertheless were unable to mature into functioning oligodendrocytes (Stolt et al., 2004b). Through promoting gliogenesis and facilitating the maturation of OPL cells, genes of the *Sox* family are thus essential for the generation of oligodendrocytes and the development of the brain as a whole.

In order to differentiate into neurons and glia, endogenous stem cells in the developing brain rely on the factors discussed above as well as many others (reviewed by Wen et al., 2009). Exogenous stem cells transplanted into the mature brain have been found to respond to stroke and demyelination via some of the same mechanisms. Yandava and colleagues were among the first to demonstrate that the engraftment of NSCs into the brains of myelin-depleted shiverer mice results in the significant remyelination and CNS axons (Yandava et al., 1999). More recent work has revealed that the use of stem cells genetically modified to express Shh (Liu et al., 2007) and Olig2 (Coprav et al., 2006; Hwang et al., 2009) for this purpose allow for more efficient remyelination, presumably through inducing transplanted NSCs to undergo the oligodendral differentiation program. It is thus apparent that therapeutic stem cells engrafted into animal models of multiple sclerosis differentiate into oligodendrocytes by mechanisms similar to those employed by endogenous NSCs.

While stem cells transplanted into the brains of animal models of stroke are known to correct for the effects of ischemia and hemorrhage, the molecular mechanisms by which this occurs are not as clear as in demyelinating disease. NSCs and MSCs engrafted in the brains of rats subjected to middle cerebral artery (MCA) occlusion have been observed to improve neurological function versus untreated controls (Veizovic et al., 2001; Chu et al., 2003; Kurozumi et al., 2004). Similar restoration of function is evident in postintracerebral hemorrhage rats treated with immortalized NSCs (Lee et al., 2007) and bone marrow-derived MSCs (Nagai et al., 2007). In all of these studies functional restoration was attributed to the differentiation of the engrafted stem cells into neurons and astrocytes; however, the molecular mediators responsible for this differentiation are at present poorly understood.

The use of MSCs for the treatment of neural lesions has not been studied as extensively as that of NSCs. Despite this relative paucity of research into therapeutic MSCs, there is evidence suggesting that they have the ability to play a significant role in therapies for neurologic diseases both alone and in combination with NSCs. Recent work by Croft and colleagues has demonstrated that genetically engineered MSCs are able to influence differentiation patterns of NSCs in coculture. Specifically, MSCs expressing the neural antigens Tuj-1 and GFAP were observed to induce the differentiation of NSCs into neuronal and astrocytic lineages, respectively (Croft and Przyborski, 2009). These results suggest that

similarly engineered MSCs may be used to fine-tune the differentiation of both endogenous and exogenous NSCs in the setting of neurologic dysfunction.

MSCs have definite potential to be used in an adjunctive capacity to NSCs; however, a number of studies have also shown that they also have the capability to be used therapeutically in the absence of cotransplanted NSCs. As cited above, MSCs have been observed to efficiently migrate to and effectively treat ischemic (Kurozumi et al., 2004), hemorrhagic (Nagai et al., 2007), and neoplastic (Lee et al., 2009; Sasportas et al., 2009) lesions in the mammalian brain. It is likely that this extensive therapeutic capability is at least in part due to the ability of exogenous MSCs to differentiate into neural stem-like cells, a phenomenon that has been reported by several groups (Deng et al., 2006; Kim et al., 2006; Yuan et al., 2006). The mechanisms underlying this “trans-differentiation” are at present poorly understood; however, recent work by Egea and colleagues has shown that tumor necrosis factor- α (TNF- α) is a powerful mediator of MSC differentiation into cells of a neural phenotype. In an elegant set of experiments this group demonstrated that long-term incubation of unaltered MSCs in low concentrations of TNF- α resulted in the development of morphologically astrocytic cells (Egea et al., 2011). Microarray analyses displayed that TNF- α induces the expression of many genes specifying a neural lineage, including *GFAP*, *MAP2*, *LIF*, *BMP2*, and *SOX2* (Egea et al., 2011). Interestingly, markers of mature oligodendrocytes and neurons, such as galactocerebro-side and β III-tubulin, respectively, were not expressed by treated cells, indicating that TNF- α specifically drives MSCs toward an astrocytic phenotype (Egea et al., 2011). The reason for this preference toward astrocytes is unclear, and while these results do represent a major step forward in terms of our understanding of exogenous stem cell differentiation, it is evident that a great deal of research is still needed before neural and mesenchymal stem cells can be used to their full therapeutic potential.

VISUALIZING MULTIPLE ASPECTS OF STEM CELL-BASED THERAPIES IN REAL TIME *IN VIVO*

It is quite obvious that before any potential human application of NSC-based therapies can be envisaged, knowledge of the location, temporospatial migration, and differentiating fate of transplanted modified NSCs will be of the utmost importance in analyzing mechanisms of correction and cell distribution. Initial studies from our laboratory have shown that NSC migration can be noninvasively followed in real time along their migratory path toward tumors after transplantation of NSC engineered with firefly luciferase (Fluc) in mouse models of glioma (Tang et al., 2003). In order to follow therapeutic NSC and changes in glioma burden in real time, we designed a study in which NSCs were genetically modified to express both S-TRAIL and Fluc and tumor cells were engineered to express *Renilla luciferase* (Rluc). The entire process of tumor formation, NSC migration, NSC dispersion throughout the tumor, and NSC killing of glioma cells was monitored noninvasively by dual bioluminescence imaging (Shah et al., 2005). In recent studies, we have shown tumor tracking of human NSCs and MSCs at a cellular resolution using hybrid (fluorescence and bioluminescence) reporter constructs and intravital imaging *in vivo* (Shah et al., 2008; Sasportas et al., 2009). This allows not only the imaging in real time of gross stem cell migration, but moreover to visualize tumor penetration by therapeutic stem cells at the single cell level. The above-mentioned capacities for tracking and monitoring of grafted NSC behavior, both longitudinally in a quantitative manner and qualitatively with high resolution, add greatly to our tool kit in the studying of stem cell biology and developing therapeutic strategy. Although stem cells are promising therapeutic delivery vehicles, pre-clinical and clinical applications of stem cell-based therapy would benefit significantly from the ability to simultaneously determine therapeutic efficacy and pharmacokinetics of therapies delivered by engineered stem cells. In a recent study, we have engineered and

screened numerous fusion variants that contained therapeutic (TRAIL) and diagnostic (luciferase) domains designed to allow simultaneous investigation of multiple events in stem cell-based therapy *in vivo*. When various stem cell lines were engineered with the optimized molecule, SRL0L2TR, diagnostic imaging showed marked differences in the levels and duration of secretion between stem cell lines, while the therapeutic activity of the molecule showed the different secretion levels translated to significant variability in tumor cell killing (Hingtgen et al., 2010). *In vivo*, simultaneous diagnostic and therapeutic monitoring revealed that stem cell-based delivery significantly improved the pharmacokinetics and antitumor effectiveness of the therapy compared with i.v. or intratumoral delivery (Hingtgen et al., 2010).

Given the need for stem cell imaging modalities in larger animals or humans (where bioluminescent and fluorescent imaging is precluded by limited depth of tissue penetration), magnetic resonance imaging (MRI) and positron emission tomography (PET) have been evaluated for feasibility of stem cell tracking. NSCs tagged with ferromagnetic material or with gadolinium rhodamine dextran have been tracked using MRI (Brekke et al., 2007). In addition to the creation of new molecularly based imaging tools, the development of novel image analytical methods have also helped to improve understanding of the dynamics of stem cell-based brain tumor therapy.

CONCLUSIONS AND CLINICAL PERSPECTIVES

Stem cells have a tremendous potential to be used in the treatment of a variety of neurologic disorders. Transplantation experiments have shown that NSCs and MSCs are capable of migrating efficiently toward neurological lesions, where they have been observed to deliver disease-specific therapeutics and spontaneously repair damaged tissue. A detailed understanding of these mechanisms would be of great value in designing strategies to boost endogenous repair and engineer exogenous stem cells to express therapeutic transgenes. However, there are many questions that must be answered before they can be used in this capacity. The ability of NSCs to differentiate into neurons and oligodendrocytes appears to be an avenue by which stroke and multiple sclerosis may eventually be treated, but before this is possible a reliable source of NSCs must be found given that at present they cannot be derived from adult tissues. The *in vitro* manipulation of mature somatic cells to induce regression toward pluripotentiality and exploitation of the plasticity of MSCs have both been proposed as alternatives to using NSCs derived from human tissues. While reasonable, further research into the feasibility of these techniques is needed. Much more pressing questions involve the safety of transplanting genetically engineered stem cells into the human brain. As with other stem cell-mediated therapies for human disease, de novo tumor formation by transplanted stem cells is of definite concern. Historically, nonimmortalized stem cells have been used safely and effectively to treat various forms of leukemia through bone marrow transplantation, and many studies concerning the use of nonimmortalized stem cells in the treatment of neuropathology in mammals have found no evidence of de novo tumorigenesis. While these results are promising, more work is needed to ensure the safety of these novel therapies before they can be extensively studied in humans.

Abbreviations

bHLH	basic helix-loop-helix
BMP	bone morphogenetic protein
CCR2	chemokine receptor 2
CXCR4	chemokine receptor 4

FGF2	fibroblast growth factor 2
HGF	hepatocyte growth factor
MCP-1	monocyte chemoattractant protein-1
MSC	mesenchymal stem cell
NSC	neural stem cell
OPL	oligodendrocyte precursor
SCF	stem cell factor
SDF-1	stromal cell-derived factor 1
Shh	sonic hedgehog
S-TRAIL	secretable form of tumor necrosis factor-related apoptosis-inducing ligand
TNF-α	tumor necrosis factor- α
TSP-1	thrombospondin-1
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

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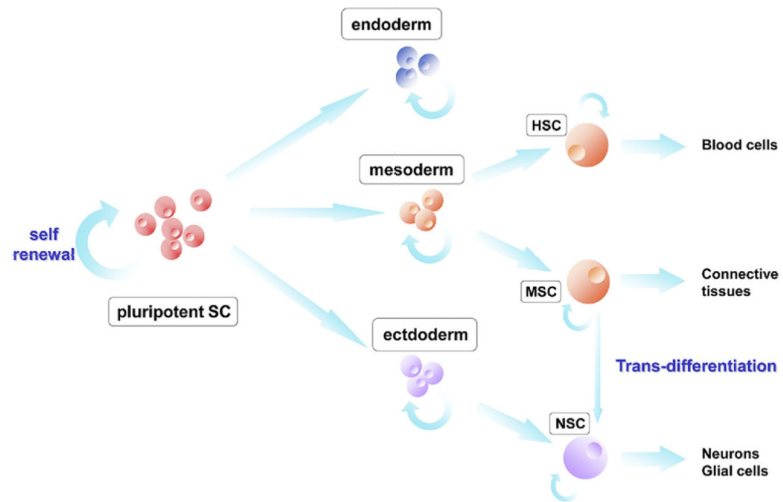


Fig. 1. Model of stem cell fate. From a pluripotent state, stem cells gradually lose breadth of differentiation potential. Note the potential for “trans-differentiation” of MSCs to NSCs. HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; NSC, neural stem cell. Adapted from Corsten and Shah (2008).

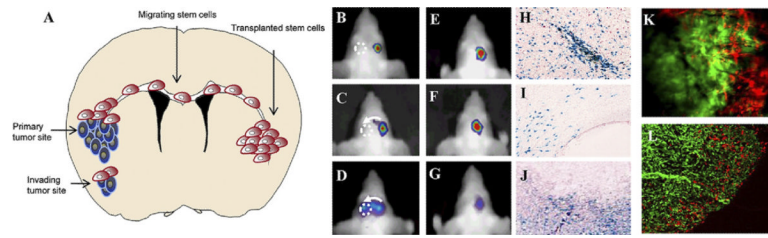


Fig. 2.

Neural stem cells migrate specifically to tumors. (A) Schematic representation showing tropism of transplanted stem cells showing for malignant cells in mouse models of brain tumors. (B–G) NSC-LUC cells were implanted into the right hemisphere of glioma-bearing (B–D) or control mice that did not have tumors (E–G). A time series of the same animal from the first group imaged on day 0 (B), at 1 wk (C), and at 2 wk (D). Migration toward the tumor (dotted circle) was first noted after 1 wk (C; see faint bioluminescence signal along arrow) and migration across the midline was evident at 2 wk. (E–G) Time series of another animal representative of the non-tumor-bearing group, in which no migration toward the contralateral side was observed. (H–J) X-Gal staining of coronal sections of the brain, showing β -Gal-expressing NSCs (blue). β -Gal-expressing cells evident at the injection site (H), the corpus callosum (I), and inside the tumor (J). (K) Intravital microscopy of Fluc-DsRed2 hNSCs implanted in mice with established GFP-Rluc gliomas. (L) Fluorescent image of showing hNSCs (red) infiltrating a tumor (green) at 40 \times magnification. Adapted from Tang et al. (2003) and Shah et al. (2008).

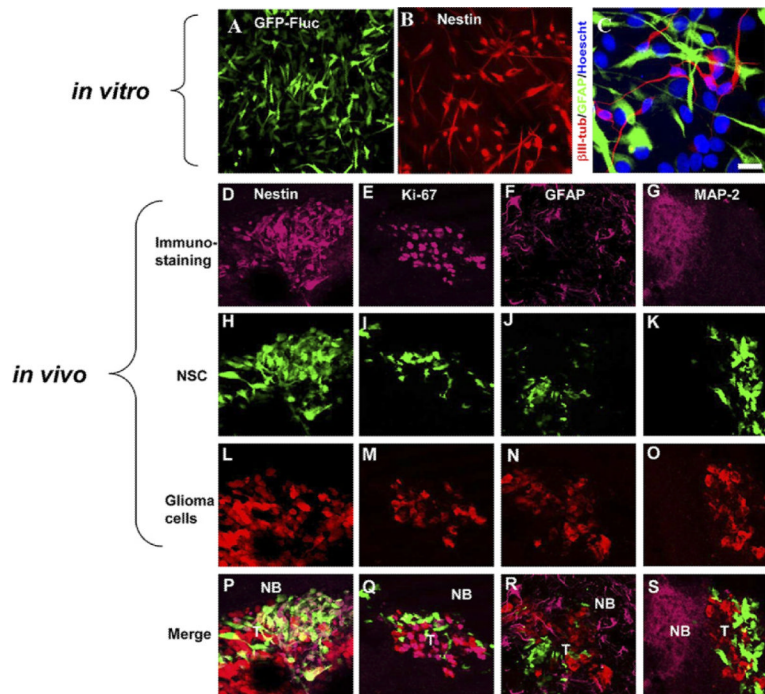


Fig. 3. Engineered human (h) NSCs do not differentiate *in vitro* or in the mouse glioma model *in vivo*. (A–C) hNSCs were differentiated for 7 d, and immunocytochemistry was performed with nestin (B) and β III-tubulin, and GFAP antibodies (C) and detected with CY3- or CY5-conjugated antibody. (D–S) hNSC-aaTSP-1 or control hNSC-GFP-Rluc were implanted in the close vicinity of established Gli36-EGFRvIII-FD gliomas. Representative images of brain sections of hNSC-aaTSP-1 mice killed on day 12 and immunostained with nestin, Ki67, GFAP, and mitogen-activated protein (MAP)-2 antibodies. Different panels showing the expression of tumor cells (red), hNSC (green), and nestin (D–E), Ki67 (H–J), GFAP (L–O), or MAP-2 (P–S) immunostaining (purple). Abbreviations: NB, normal brain; T, tumor. Adapted from Shah et al. (2008) and van Eekelen et al. (2010).

Table 1

Receptor/ligand pairs implicated in NSC pathotropism

Receptor/ligand	Disease model	References
SCF/c-Kit	"Freeze" brain injury	Sun et al. (2004)
SCF/c-Kit	Huntington's disease	Bantubungi et al. (2008)
MCP-1/CCR2	Inflammation	Widera et al. (2004)
MCP-1/CCR2	Tumor	Magge et al. (2009)
VEGF/VEGFR	Ischemia	Xu et al. (2007)
		Zhao et al. (2008)
		Schmidt et al. (2009)
HGF/c-Met	Tumor	Heese et al. (2005)
SDF-1/CXCR4	Demyelination	Carbajal et al. (2010)
SDF-1/CXCR4	Ischemia	Imitola et al. (2004)
SDF-1/CXCR4	Tumor	Zhou et al. (2002)
		Robin et al. (2006)