Stem Cell Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.

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Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Alexanian, A. R., et al. (2008). "In vitro and in vivo characterization of neurally modified mesenchymal stem cells induced by epigenetic modifiers and neural stem cell environment." Stem Cells Dev **17**(6): 1123-1130.

Mesenchymal stem cell (MSC)-mediated tissue regeneration is a promising strategy to treat several neurodegenerative diseases and traumatic injuries of the central nervous system. Bone marrow MSCs have great potential as therapeutic agents, since they are easy to isolate and expand and are capable of producing various cell types, including neural cells. Recently we developed a highly efficient methodology to produce neural stem-like and neural precursor-like cells from mice bone marrow-derived MSCs that eventually differentiate into neuronal- and glial-like cells in vitro. The aim of this study is to further elucidate neural expression profile of neurally induced mesenchymal stem cells (NI-MSCs) and their ability to retain neural differentiation potential when grafted into the intact spinal cord of rats. To this end, we further characterized in vitro and in vivo properties of NI-MSCs by immunocytochemistry, Western blot, ELISA, and immunohistochemistry. Immunocytochemical data demonstrated that NI-MSCs express several mature neural markers such as B3T, GFAP MAP-2, NF-200, and NeuN, which were confirmed through Western blot. ELISA data showed that NI-MSCs release nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). In vivo studies demonstrated that grafted NI-MSCs survived after transplantation into intact spinal cord and produced cells that expressed neural markers. All these data suggest that neurally modified MSCs, induced by recently developed methodology, could be a potential source of cells to replace damaged neurons and glia in injured spinal cord, and/or to promote cell survival and axonal growth of host tissue.

Babenko, V. A., et al. (2018). "Miro1 Enhances Mitochondria Transfer from Multipotent Mesenchymal Stem Cells (MMSC) to Neural Cells and Improves the Efficacy of Cell Recovery." Molecules **23**(3).

A recently discovered key role of reactive oxygen species (ROS) in mitochondrial traffic has opened a wide alley for studying the interactions between cells, including stem cells. Since its discovery in 2006, intercellular mitochondria transport has been intensively studied in different cellular models as a basis for cell therapy, since the

potential of replacing malfunctioning organelles appears to be very promising. In this study, we explored the transfer of mitochondria from multipotent mesenchymal stem cells (MMSC) to neural cells and analyzed its efficacy under normal conditions and upon induction of mitochondrial damage. We found that mitochondria were transferred from the MMSC to astrocytes in a more efficient manner when the astrocytes were exposed to ischemic damage associated with elevated ROS levels. Such transport of mitochondria restored the bioenergetics of the recipient cells and stimulated their proliferation. The introduction of MMSC with overexpressed Miro1 in animals that had undergone an experimental stroke led to significantly improved recovery of neurological functions. Our data suggest that mitochondrial impairment in differentiated cells can be compensated by receiving healthy mitochondria from MMSC. We demonstrate a key role of Miro1, which promotes the mitochondrial transfer from MMSC and suggest that the genetic modification of stem cells can improve the therapies for the injured brain.

Bai, L., et al. (2007). "Human mesenchymal stem cells signals regulate neural stem cell fate." Neurochem Res **32**(2): 353-362.

Neural stem cells (NSCs) differentiate into neurons, astrocytes and oligodendrocytes depending on their location within the central nervous system (CNS). The cellular and molecular cues mediating end-stage cell fate choices are not completely understood. The retention of multipotent NSCs in the adult CNS raises the possibility that selective recruitment of their progeny to specific lineages may facilitate repair in a spectrum of neuropathological conditions. Previous studies suggest that adult human bone marrow derived mesenchymal stem cells (hMSCs) improve functional outcome after a wide range of CNS insults, probably through their trophic influence. In the context of such trophic activity, here we demonstrate that hMSCs in culture provide humoral signals that selectively promote the genesis of neurons and oligodendrocytes from NSCs. Cell-cell contacts were less effective and the proportion of hMSCs that could be induced to express neural characteristics was very small. We propose that the selective promotion of neuronal and oligodendroglial fates in neural stem cell progeny is responsible for the ability of MSCs to enhance recovery after a wide range of CNS injuries.

Chen, G., et al. (2013). "Neural stem cell-like cells derived from autologous bone mesenchymal stem cells for the treatment of patients with cerebral palsy." J Transl Med **11**: 21.

BACKGROUND: Stem cell therapy is a promising treatment for cerebral palsy, which refers to a category of brain diseases that are associated with chronic motor disability in children. Autologous MSCs may be a better cell source and have been studied for the treatment of cerebral palsy because of their functions in tissue repair and the regulation of immunological processes. METHODS: To assess neural stem cell-like (NSC-like) cells derived from autologous marrow mesenchymal stem cells as a novel treatment for patients with moderate-to-severe cerebral palsy, a total of 60 cerebral palsy patients were enrolled in this open-label, non-randomised, observer-blinded controlled clinical study with a 6-months follow-up. For the transplantation group, a total of 30 cerebral palsy patients received an autologous NSC-like cells transplantation (1-2 x 107 cells into the subarachnoid cavity) and rehabilitation treatments whereas 30 patients in the control group only received rehabilitation treatment. RESULTS: We recorded the gross motor function measurement scores, language quotients, and adverse events up to 6 months post-treatment. The gross motor function measurement scores in the transplantation group were significantly higher at month 3 (the score increase was 42.6, 95% CI: 9.8-75.3, P=.011) and month 6 (the score increase was 58.6, 95% CI: 25.8-91.4, P=.001) post-treatment compared with the baseline scores. The increase in the Gross Motor Function Measurement scores in the control group was not significant. The increases in the language quotients at months 1, 3, and 6 post-treatment were not statistically significant when compared with the baseline quotients in both groups. All the 60 patients survived, and none of the patients experienced serious adverse events or complications. CONCLUSION: Our results indicated that NSC-like cells are safe and effective for the treatment of motor deficits related to cerebral palsy. Further randomised clinical trials are necessary to establish the efficacy of this procedure.

Chijimatsu, R., et al. (2017). "Characterization of Mesenchymal Stem Cell-Like Cells Derived From Human iPSCs via Neural Crest Development and Their Application for Osteochondral Repair." Stem Cells Int **2017**: 1960965.

Mesenchymal stem cells (MSCs) derived from induced pluripotent stem cells (iPSCs) are a promising cell source for the repair of skeletal disorders. Recently, neural crest cells (NCCs) were reported to be effective for inducing mesenchymal progenitors, which have potential to differentiate into

osteochondral lineages. Our aim was to investigate the feasibility of MSC-like cells originated from iPSCs via NCCs for osteochondral repair. Initially, MSC-like cells derived from iPSC-NCCs (iNCCs) were generated and characterized in vitro. These iNCC-derived MSC-like cells (iNCMSCs) exhibited a homogenous population and potential for osteochondral differentiation. No upregulation of pluripotent markers was detected during culture. Second, we implanted iNCMSC-derived tissue-engineered constructs into rat osteochondral defects without any preinduction for specific differentiation lineages. The implanted cells remained alive at the implanted site, whereas they failed to repair the defects, with only scarce development of osteochondral tissue in vivo. With regard to tumorigenesis, the implanted cells gradually disappeared and no malignant cells were detected throughout the 2-month follow-up. While this study did not show that iNCMSCs have efficacy for repair of osteochondral defects when implanted under undifferentiated conditions, iNCMSCs exhibited good chondrogenic potential in vitro under appropriate conditions. With further optimization, iNCMSCs may be a new source for tissue engineering of cartilage.

Croft, A. P. and S. A. Przyborski (2009). "Mesenchymal stem cells expressing neural antigens instruct a neurogenic cell fate on neural stem cells." Exp Neurol **216**(2): 329-341.

The neurogenic response to injury in the postnatal brain is limited and insufficient for restoration of function. Recent evidence suggests that transplantation of mesenchymal stem cells (MSCs) into the injured brain is associated with improved functional recovery, mediated in part through amplification in the endogenous neurogenic response to injury. In the current study we investigate the interactions between bone marrow-derived MSCs and embryonic neural stem cells (NSCs) plus their differentiated progeny using an in vitro co-culture system. Two populations of MSCs were used, MSCs induced to express neural antigens (nestin+, Tuj-1+, GFAP+) and neural antigen negative MSCs. Following co-culture of induced MSCs with differentiating NSC/progenitor cells a significant increase in Tuj-1+ neurons was detected compared to co-cultures of non-induced MSCs in which an increase in astrocyte (GFAP+) differentiation was observed. The effect was mediated by soluble interactions between the two cell populations and was independent of any effect on cell death and proliferation. Induced and non-induced MSCs also promoted the survival of Tuj-1+ cell progeny in long-term cultures and both promoted axonal growth, an effect also seen in differentiating neuroblastoma cells. Therefore, MSCs provide instructive signals that are able to direct the differentiation of NSCs and promote axonal development in neuronal progeny. The data indicates that the nature of MSC derived signals is dependent not only on their microenvironment but on the developmental status of the MSCs. Pre-manipulation of MSCs prior to transplantation in vivo may be an effective means of enhancing the endogenous neurogenic response to injury.

Dong, B. T., et al. (2015). "Lithium enhanced cell proliferation and differentiation of mesenchymal stem cells to neural cells in rat spinal cord." Int J Clin Exp Pathol **8**(3): 2473-2483.

Lithium has been shown to inhibit apoptosis of neural progenitor cells (NPCs) and promote differentiation of NPCs. However, there was rare data to discuss the effects of lithium on neural differentiation of mesenchymal stem cells (MSCs). Here, we investigated the potential promotion of lithium to MSC proliferation and neural differentiation in vitro and after transplanted into the ventral horn of rat spinal cord in vivo. We found that lithium possesses the ability to promote proliferation of GFP-MSCs in a dose dependent manner as verified by growth curve and bromodeoxyuridine (BrdU) incorporation assays; While in neural induction medium, lithium (0.1 mM) promotes neural differentiation of GFP-MSCs as verified by immunostaining and quantitative analysis. After transplantation of GFP-MSCs into the rat spinal cord, lithium treatment enhanced cell survival and neural differentiation after transplantation as verified by immunohistochemistry. These data suggested that lithium could be a potential drug to augment the therapeutic efficiency of MSCs transplantation therapy in central nervous system (CNS) disorders.

Fang, H., et al. (2018). "Bone mesenchymal stem cell-conditioned medium decreases the generation of astrocytes during the process of neural stem cells differentiation." J Spinal Cord Med **41**(1): 10-16.

OBJECTIVES: The aim of this study was to investigate the effect of bone mesenchymal stem cell (BMSC) conditioned medium (CM) and Bone morphogenetic protein-4 (BMP-4) on the generation of astrocytes during the process of NSCs differentiation. DESIGN: Neural stem cells (NSCs) were grown under different culture conditions. SETTING: The First Affiliated Hospital of Anhui Medical University, Hefei, China. OUTCOME MEASURES: The study consisted of four groups:

NSCs cultured under control conditions (group 1) or with the addition of BMSC-CM (group 2);(BMP-4) (group 3) or both (group 4).The expression of glial fibrillary acidic protein (GFAP) was detected by immunocytochemical staining and Western blotting. RESULTS: The expression of GFAP was higher in Group3 and lower in Group 2 compared to that in Group 1. The expression of GFAP in Group 4 was intermediate between that of Group 2 and Group 3. CONCLUSIONS: These results suggest that BMSC-CM can decrease the generation of astrocytes and that the inhibition of the (BMP-4) /Smad1/5/8 signaling pathway may be the underlying mechanism. This phenomenon may be mediated by increasing the expression of Smad6.

Fu, L., et al. (2008). "Derivation of neural stem cells from mesenchymal stemcells: evidence for a bipotential stem cell population." Stem Cells Dev **17**(6): 1109-1121.

Neural stem cell (NSC) transplantation has been proposed as a future therapy for neurodegenerative disorders. However, NSC transplantation will be hampered by the limited number of brain donors and the toxicity of immunosuppressive regimens that might be needed with allogeneic transplantation. These limitations may be avoided if NSCs can be generated from clinically accessible sources, such as bone marrow (BM) and peripheral blood samples, that are suitable for autologous transplantation. We report here that NSCs can be generated from human BM-derived mesenchymal stem cells (MSCs). When cultured in NSC culture conditions, 8% of MSCs were able to generate neurospheres. These MSC-derived neurospheres expressed characteristic NSC antigens, such as nestin and musashi-1, and were capable of self-renewal and multilineage differentiation into neurons, astrocytes, and oligodendrocytes. Furthermore, when these MSC-derived neurospheres were cocultured with primary astrocytes, they differentiate into neurons that possess both dendritic and axonal processes, form synapses, and are able to fire tetrodotoxin-sensitive action potentials. When these MSC-derived NSCs were switched back to MSC culture conditions, a small fraction of NSCs (averaging 4-5%) adhered to the culture flasks, proliferated, and displayed the morphology of MSCs. Those adherent cells expressed the characteristic MSC antigens and regained the ability to differentiate into multiple mesodermal lineages. Data presented in this study suggest that MSCs contain a small fraction (averaging 4-5%) of a bipotential stem cell population that is able to generate either MSCs or NSCs depending on the culture conditions.

Harris, V. K., et al. (2012). "Characterization of autologous mesenchymal stem cell-derived neural progenitors as a feasible source of stem cells for central nervous system applications in multiple sclerosis." Stem Cells Transl Med **1**(7): 536-547.

Bone marrow mesenchymal stem cell-derived neural progenitors (MSC-NPs) are a potential therapeutic source of cells that have been shown to be efficacious in a preclinical model of multiple sclerosis (MS). To examine the feasibility of using MSC-NPs as an autologous source of cells to promote central nervous system (CNS) repair in MS, this study characterized human MSC-NPs from a panel of both MS and non-MS donors. Expanded MSCs showed similar characteristics in terms of growth and cell surface phenotype, regardless of the donor disease status. MSC-NPs derived from all MSCs showed a consistent pattern of gene expression changes that correlated with neural commitment and increased homogeneity. Furthermore, the reduced expression of mesodermal markers and reduced capacity for adipogenic or osteogenic differentiation in MSC-NPs compared with MSCs suggested that MSC-NPs have reduced potential of unwanted mesodermal differentiation upon CNS transplantation. The immunoregulatory function of MSC-NPs was similar to that of MSCs in their ability to suppress T-cell proliferation and to promote expansion of FoxP3-positive T regulatory cells in vitro. In addition, MSC-NPs promoted oligodendroglial differentiation from brain-derived neural stem cells that correlated with the secretion of bioactive factors. Our results provide a set of identity characteristics for autologous MSC-NPs and suggest that the in vitro immunoregulatory and trophic properties of these cells may have therapeutic value in the treatment of MS.

Hill, A. J., et al. (2009). "Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after intravitreal grafting in neonatal rats." Stem Cells Dev **18**(3): 399-409.

This study investigated the ability of mesenchymal stem cells (MSCs) derived from full-term human umbilical cord blood to survive, integrate and differentiate after intravitreal grafting to the degenerating neonatal rat retina following intracranial optic tract lesion. MSCs survived for 1 week in the absence of immunosuppression. When host animals were treated with cyclosporin A and dexamethasone to suppress inflammatory and immune responses, donor cells survived for at least 3 weeks, and were able to spread and cover the entire vitreal surface of the host retina. However, MSCs did

not significantly integrate into or migrate through the retina. They also maintained their human antigenicity, and no indication of neural differentiation was observed in retinas where retinal ganglion cells either underwent severe degeneration or were lost. These results have provided the first in vivo evidence that MSCs derived from human umbilical cord blood can survive for a significant period of time when the host rat response is suppressed even for a short period. These results, together with the observation of a lack of neuronal differentiation and integration of MSCs after intravitreal grafting, has raised an important question as to the potential use of MSCs for neural repair through the replacement of lost neurons in the mammalian retina and central nervous system.

Hosseini, S. M., et al. (2015). "Combination cell therapy with mesenchymal stem cells and neural stem cells for brain stroke in rats." Int J Stem Cells **8**(1): 99-105.

OBJECTIVES: Brain stroke is the second most important events that lead to disability and morbidity these days. Although, stroke is important, there is no treatment for curing this problem. Nowadays, cell therapy has opened a new window for treating central nervous system disease. In some previous studies the Mesenchymal stem cells and neural stem cells. In this study, we have designed an experiment to assess the combination cell therapy (Mesenchymal and Neural stem cells) effects on brain stroke. METHOD AND MATERIALS: The Mesenchymal stem cells were isolated from adult rat bone marrow and the neural stem cells were isolated from ganglion eminence of rat embryo 14 days. The Mesenchymal stem cells were injected 1 day after middle cerebral artery occlusion (MCAO) and the neural stem cells transplanted 7 day after MCAO. After 28 days, the neurological outcomes and brain lesion volumes were evaluated. Also, the activity of Caspase 3 was assessed in different groups. RESULT: The group which received combination cell therapy had better neurological examination and less brain lesion. Also the combination cell therapy group had the least Caspase 3 activity among the groups. CONCLUSIONS: The combination cell therapy is more effective than Mesenchymal stem cell therapy and neural stem cell therapy separately in treating the brain stroke in rats.

Hosseini, S. M., et al. (2018). "Concomitant use of mesenchymal stem cells and neural stem cells for treatment of spinal cord injury: A combo cell therapy approach." Neurosci Lett **668**: 138-146.

TREATMENT: with neural stem cells (NSCs) provides a hope to recover the neural damage and compensate for the lost neural structures for restoration of interrupted neural communications above and below the site of injury. However, cell-based therapy approach suffers from many biological barriers and technical caveats which severely hamper the prognosis. The biochemically-rich microenvironment at the site of spinal cord injury (SCI), the continuing neuro-degenerative process and infiltrating immune cells offer a serious barrier to the donor cells. We hypothesized that mesenchymal stem cells (MSCs) concomitantly delivered with NSCs would significantly enhance the effectiveness of cell-based therapy for SCI. In a rodent model of SCI (n=15 animals/group), MSCs labeled with PKH67 (green fluorescence dye) were delivered on day1 after SCI whereas the same animals were treated with NSCs during the subacute phase on day3 (group-5). In comparison with untreated control (group-1), sham group (without cell treatment; group-2), MSCs alone (group-3) and NSCs alone treated animals (group-4), the combined cell treated animals (group-5) showed significantly higher homing of cells at the site of injury during in vivo imaging. Caspase-3 activity was lower in group-5 ($P<0.05$ vs all groups) with concomitant reduction in the pro-inflammatory cytokines IL-1beta and IL-6 ($P<0.05$ vs all groups). All cell therapy groups showed significant improvement in neurological function as compared to group-2, however, it was highest in group-5 ($P<0.05$ vs all groups). In conclusion, combined treatment with (NSCs+MSCs) enhances NSCs survival and functional recovery in SCI and is superior to the treatment with either of NSCs or MSCs alone.

Huat, T. J., et al. (2014). "IGF-1 enhances cell proliferation and survival during early differentiation of mesenchymal stem cells to neural progenitor-like cells." BMC Neurosci **15**: 91.

BACKGROUND: There has been increasing interest recently in the plasticity of mesenchymal stem cells (MSCs) and their potential to differentiate into neural lineages. To unravel the roles and effects of different growth factors in the differentiation of MSCs into neural lineages, we have differentiated MSCs into neural lineages using different combinations of growth factors. Based on previous studies of the roles of insulin-like growth factor 1 (IGF-1) in neural stem cell isolation in the laboratory, we hypothesized that IGF-1 can enhance proliferation and reduce apoptosis in neural progenitor-like cells (NPCs) during differentiation of MSCs into NCPs. We induced MSCs differentiation under four different combinations of growth factors: (A) EGF + bFGF, (B) EGF + bFGF + IGF-1, (C) EGF + bFGF + LIF, (D) EGF + bFGF + BDNF, and (E) without

growth factors, as a negative control. The neurospheres formed were characterized by immunofluorescence staining against nestin, and the expression was measured by flow cytometry. Cell proliferation and apoptosis were also studied by MTS and Annexin V assay, respectively, at three different time intervals (24 hr, 3 days, and 5 days). The neurospheres formed in the four groups were then terminally differentiated into neuron and glial cells. RESULTS: The four derived NPCs showed a significantly higher expression of nestin than was shown by the negative control. Among the groups treated with growth factors, NPCs treated with IGF-1 showed the highest expression of nestin. Furthermore, NPCs derived using IGF-1 exhibited the highest cell proliferation and cell survival among the treated groups. The NPCs derived from IGF-1 treatment also resulted in a better yield after the terminal differentiation into neurons and glial cells than that of the other treated groups. CONCLUSIONS: Our results suggested that IGF-1 has a crucial role in the differentiation of MSCs into neuronal lineage by enhancing the proliferation and reducing the apoptosis in the NPCs. This information will be beneficial in the long run for improving both cell-based and cell-free therapy for neurodegenerative diseases.

Isern, J., et al. (2014). "The neural crest is a source of mesenchymal stem cells with specialized hematopoietic stem cell niche function." Elife **3**: e03696.

Mesenchymal stem cells (MSCs) and osteolineage cells contribute to the hematopoietic stem cell (HSC) niche in the bone marrow of long bones. However, their developmental relationships remain unclear. In this study, we demonstrate that different MSC populations in the developing marrow of long bones have distinct functions. Proliferative mesoderm-derived nestin(-) MSCs participate in fetal skeletogenesis and lose MSC activity soon after birth. In contrast, quiescent neural crest-derived nestin(+) cells preserve MSC activity, but do not generate fetal chondrocytes. Instead, they differentiate into HSC niche-forming MSCs, helping to establish the HSC niche by secreting Cxcl12. Perineural migration of these cells to the bone marrow requires the ErbB3 receptor. The neonatal Nestin-GFP(+) Pdgfralpha(-) cell population also contains Schwann cell precursors, but does not comprise mature Schwann cells. Thus, in the developing bone marrow HSC niche-forming MSCs share a common origin with sympathetic peripheral neurons and glial cells, and ontogenically distinct MSCs have non-overlapping functions in endochondrogenesis and HSC niche formation.

Jang, S., et al. (2015). "Neural-induced human mesenchymal stem cells promote cochlear cell regeneration in deaf Guinea pigs." Clin Exp Otorhinolaryngol **8**(2): 83-91.

OBJECTIVES: In mammals, cochlear hair cell loss is irreversible and may result in a permanent sensorineural hearing loss. Secondary to this hair cell loss, a progressive loss of spiral ganglion neurons (SGNs) is presented. In this study, we have investigated the effects of neural-induced human mesenchymal stem cells (NI-hMSCs) from human bone marrow on sensory neuronal regeneration from neomycin treated deafened guinea pig cochleae. METHODS: HMSCs were isolated from the bone marrow which was obtained from the mastoid process during mastoidectomy for ear surgery. Following neural induction with basic fibroblast growth factor and forskolin, we studied the several neural marker and performed electrophysiological analysis. NI-hMSCs were transplanted into the neomycin treated deafened guinea pig cochlea. Engraftment of NI-hMSCs was evaluated immunohistologically at 8 weeks after transplantation. RESULTS: Following neural differentiation, hMSCs expressed high levels of neural markers, ionic channel markers, which are important in neural function, and tetrodotoxin-sensitive voltage-dependent sodium currents. After transplantation into the scala tympani of damaged cochlea, NI-hMSCs-injected animals exhibited a significant increase in the number of SGNs compared to Hanks balanced salt solution-injected animals. Transplanted NI-hMSCs were found within the perilymphatic space, the organ of Corti, along the cochlear nerve fibers, and in the spiral ganglion. Furthermore, the grafted NI-hMSCs migrated into the spiral ganglion where they expressed the neuron-specific marker, NeuN. CONCLUSION: The results show the potential of NI-hMSCs to give rise to replace the lost cochlear cells in hearing loss mammals.

Kanemura, Y. (2010). "Development of cell-processing systems for human stem cells (neural stem cells, mesenchymal stem cells, and iPS cells) for regenerative medicine." Keio J Med **59**(2): 35-45.

Regenerative medicine using human stem cells is one of the newest and most promising fields for treating various intractable diseases and damaged organs. For clinical applications, choosing which human stem cells to use, i.e. according to tissue of origin and progenitor type, is a critical issue. Neural stem/progenitor cells (NSPCs) hold promise for treating various neurological diseases. We have shown that the transporter protein ABCB1 is

predominantly expressed in immature human fetal NSPCs, and thus could be used as a phenotypic marker to investigate and monitor NSPCs in culture. We describe our proposed model for the in vitro proliferative process of aggregated human NSPCs and show that neurosphere enlargement and NSPC proliferation are mutually reinforcing. We have established that human neurospheres contain a heterogeneous cell population, knowledge that will contribute to the development of human neurospheres with desirable characteristics for clinical applications. Furthermore, decidua-derived mesenchymal cells (DMCs), which we isolated from human placenta, have unique properties as mesenchymal stem cells. They also generate a pericellular matrix (PCM-DM) that supports the growth and pluripotency of human embryonic stem cells and induced pluripotent stem cells (hiPS) cells. The newly developed re-programming techniques for generating hiPS cells should greatly contribute to cell therapies using human pluripotent stem cells, including those derived from DMCs. Our DMC-derived hiPS cells are a promising candidate source of allogeneic hiPS cells for clinical applications. We hope our findings will contribute to the development of cell-culture systems for generating human allogeneic stem cells for clinical use in regenerative medicine.

Karaoz, E., et al. (2011). "Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells." Histochem Cell Biol **136**(4): 455-473.

Dental pulp stem cells (hDP-SCs) were primarily derived from pulp tissues of primary incisors, exfoliated deciduous and permanent third molar teeth. To understand the characteristics of hDP-SCs from impacted third molar, proliferation capacities, gene expression profiles, phenotypic, ultrastructural, and differentiation characteristics were analyzed in comparison with human bone marrow-derived mesenchymal stem cells (hBM-MSCs), extensively. hDP-SCs showed more developed and metabolically active cells. Contrary to hBM-MSCs, hDP-SCs strongly expressed both cytokeratin (CK)-18 and -19, which could involve in odontoblast differentiation and dentine repair. The intrinsic neuro-glia characteristics of hDP-MSCs were demonstrated by the expression of several specific transcripts and proteins of neural stem cell and neurons. These cells not only differentiate into adipogenic, osteogenic, and chondrogenic lineage, but also share some special characteristics of expressing some neural stem cell and epithelial markers. Under defined conditions, hDP-SCs are able to differentiate into both neural and vascular endothelial cells in vitro. Dental pulp might provide an alternative source for human MSCs. hDP-SCs with a promising differentiation capacity could be easily isolated, and possible clinical use could be developed for neurodegenerative and oral diseases in the future.

Lamoury, F. M., et al. (2006). "Undifferentiated mouse mesenchymal stem cells spontaneously express neural and stem cell markers Oct-4 and Rex-1." Cytotherapy **8**(3): 228-242.

BACKGROUND: Previous adult stem cells studies have provided evidence that BM mesenchymal stem cells (MSC) exhibit multilineage differentiation capacity. These properties of MSC prompted us to explore the neural potential of MSC with a view to their use for the treatment of demyelinating disorders, such as multiple sclerosis. Indeed, issues such as the identification of a subset of stem cells that is neurally fated, methods of expansion and optimal stage of differentiation for transplantation remain poorly understood. METHODS: In order to isolate mouse (m) MSC from BM, we used and compared the classic plastic-adhesion method and one depleting technique, the magnetic-activated cell sorting technique. RESULTS: We established and optimized culture conditions so that mMSC could be expanded for more than 360 days and 50 passages. We also demonstrated that undifferentiated mMSC express the neural markers nestin, MAP2, A2B5, GFAP, MBP, CNPase, GalC, O1 under standard culture conditions before transplantation. The pluripotent stem cell marker Oct-4 and the embryonic stem cell marker Rex-1 are spontaneously expressed by untreated mMSC. The lineage-negative mMSC (CD5- CD11b- Ly-6G-Ter119- CD45R- c-kit/CD117-) overexpressed Oct-4, O1 and A2B5 in the first days of culture compared with the non-sorted MSC. Finally, we identified a distinct subpopulation of mMSC that is primed towards a neural fate, namely Sca-1+/nestin+ mMSC. DISCUSSION: These results should facilitate the optimal timing of harvesting a neurally fated subpopulation of mMSC for transplantation into animal models of human brain diseases.

Lee, H., et al. (2016). "Gelatin Directly Enhances Neurogenic Differentiation Potential in Bone Marrow-Derived Mesenchymal Stem Cells Without Stimulation of Neural Progenitor Cell Proliferation." DNA Cell Biol **35**(9): 530-536.

Gelatin has been reported to induce generation of mesenchymal stem cells (MSCs) with enhanced potential of differentiation into neuronal

lineage cells. However, the presence of various cell types besides MSCs in bone marrow has raised doubts about the effects of gelatin. In the following report, we determined whether gelatin can directly enhance neurogenic differentiation potential in MSCs without proliferation of neural progenitor cells (NPCs). MSCs comprised a high proportion of bone marrow-derived primary cells (BMPCs) and gelatin induced significant increases in MSC proliferation during primary culture, and the proportion of MSCs was maintained at more than 99% throughout the subculture. However, NPCs comprised a low percentage of BMPCs and a decrease in proliferation was detected despite gelatin treatment during the primary culture, and the proportion of subcultured NPCs gradually decreased. In a similar manner, MSCs exposed to gelatin during primary culture showed more enhanced neurogenic differentiation ability than those not exposed to gelatin. Together, these results demonstrate that gelatin directly enhances neurogenic differentiation in bone marrow-derived MSCs without stimulating NPC proliferation.

Li, Z., et al. (2014). "Transplantation of placenta-derived mesenchymal stem cell-induced neural stem cells to treat spinal cord injury." Neural Regen Res **9**(24): 2197-2204.

Because of their strong proliferative capacity and multi-potency, placenta-derived mesenchymal stem cells have gained interest as a cell source in the field of nerve damage repair. In the present study, human placenta-derived mesenchymal stem cells were induced to differentiate into neural stem cells, which were then transplanted into the spinal cord after local spinal cord injury in rats. The motor functional recovery and pathological changes in the injured spinal cord were observed for 3 successive weeks. The results showed that human placenta-derived mesenchymal stem cells can differentiate into neuron-like cells and that induced neural stem cells contribute to the restoration of injured spinal cord without causing transplant rejection. Thus, these cells promote the recovery of motor and sensory functions in a rat model of spinal cord injury. Therefore, human placenta-derived mesenchymal stem cells may be useful as seed cells during the repair of spinal cord injury.

Lo Furno, D., et al. (2018). "Neural differentiation of human adipose-derived mesenchymal stem cells induced by glial cell conditioned media." J Cell Physiol **233**(10): 7091-7100.

Adipose-derived mesenchymal stem cells (ASCs) may transdifferentiate into cells belonging to mesodermal, endodermal, and ectodermal lineages. The aim of this study was to verify whether a neural differentiation of ASCs could be induced by a conditioned medium (CM) obtained from cultures of olfactory ensheathing cells (OECs) or Schwann cells (SCs). ASCs were isolated from the stromal vascular fraction of adipose tissue and expanded for 2-3 passages. They were then cultured in OEC-CM or SC-CM for 24 hr or 7 days. At each stage, the cells were tested by immunocytochemistry and flow cytometer analysis to evaluate the expression of typical neural markers such as Nestin, PGP 9.5, MAP2, Synapsin I, and GFAP. Results show that both conditioned media induced similar positive effects, as all tested markers were overexpressed, especially at day 7. Overall, an evident trend toward neuronal or glial differentiation was not clearly detectable in many cases. Nevertheless, a higher tendency toward a neuronal phenotype was recognized for OEC-CM (considering MAP2 increases). On the other hand, SC-CM would be responsible for a more marked glial induction (considering GFAP increases). These findings confirm that environmental features can induce ASCs toward a neural differentiation, either as neuronal or glial elements. Rather than supplementing the culture medium by adding chemical agents, a "more physiological" condition was obtained here by means of soluble factors (cytokines/growth factors) likely released by glial cells. This culture strategy may provide valuable information in the development of cell-based therapeutic approaches for pathologies affecting the central/peripheral nervous system.

Long, X., et al. (2005). "Neural cell differentiation in vitro from adult human bone marrow mesenchymal stem cells." Stem Cells Dev **14**(1): 65-69.

Bone marrow (BM) mesenchymal stem cells (MSCs) are cells capable of expanding and differentiating in vitro into nonhematopoietic cells. Neurotrophic cytokines, such as human epidermal growth factor (hEGF) and bovine fibroblast growth factor (bFGF) can induce differentiation into neural cells (NCs). When BM MSCs were cultured with hEGF and bFGF, RNA expression of neuronal specific markers Nestin, MAP-2, and tyrosine hydroxylase (TH) were observed. We tested a new cytokine combination to generate mature NCs. The plastic-adherent cells were collected and then split when they were 90% confluent from an enriched mononuclear cell layer. At passage 3, MSCs were cultured in neural differentiation media (dbcAMP, IBMX, FGF-8, BDNF, hEGF, and bFGF in NEUROBASAL media plus B27). Cells were counted on day 6. Immunofluorescent staining and reverse transcriptase (RT)-PCR were performed to

evaluate the expression of neural markers. On day 6, 66% of cells developed dendrites and presented typical neural cell morphology. Some cells were positive for early neural markers Nestin and beta-tubulin III. Cells expressing mature neuronal markers (NF, NeuN, Tau, Nurr1, GABA, oligodendryte GalC, and glial GFAP) were also seen. By adding hEGF, bFGF, dbcAMP, IBMX, BDNF, and bFGF-8 into NEUROBASAL media plus B27, BM MSCs were directed toward becoming early and mature NCs.

Ma, K., et al. (2011). "Generation of neural stem cell-like cells from bone marrow-derived human mesenchymal stem cells." Neurol Res **33**(10): 1083-1093.

Under appropriate culture conditions, bone marrow (BM)-derived mesenchymal stem cells are capable of differentiating into diverse cell types unrelated to their phenotypical embryonic origin, including neural cells. Here, we report the successful generation of neural stem cell (NSC)-like cells from BM-derived human mesenchymal stem cells (hMSCs). Initially, hMSCs were cultivated in a conditioned medium of human neural stem cells. In this culture system, hMSCs were induced to become NSC-like cells, which proliferate in neurosphere-like structures and express early NSC markers. Like central nervous system-derived NSCs, these BM-derived NSC-like cells were able to differentiate into cells expressing neural markers for neurons, astrocytes, and oligodendrocytes. Whole-cell patch clamp recording revealed that neuron-like cells, differentiated from NSC-like cells, exhibited electrophysiological properties of neurons, including action potentials. Transplantation of NSC-like cells into mouse brain confirmed that these NSC-like cells retained their capability to differentiate into neuronal and glial cells in vivo. Our data show that multipotent NSC-like cells can be efficiently produced from BM-derived hMSCs in culture and that these cells may serve as a useful alternative to human neural stem cells for potential clinical applications such as autologous neuroreplacement therapies.

Minguell, J. J., et al. (2005). "Nonstimulated human uncommitted mesenchymal stem cells express cell markers of mesenchymal and neural lineages." Stem Cells Dev **14**(4): 408-414.

Ex vivo cultures of human bone marrow-derived mesenchymal stem cells (MSCs) contain subsets of progenitors exhibiting dissimilar properties. One of these subsets comprises uncommitted progenitors displaying distinctive features, such as morphology, a quiescent condition, growth factor production, and restricted tissue biodistribution after transplantation. In this study, we assessed the competence of these cells to express, in the absence of differentiation stimuli, markers of mesoderm and ectodermic (neural) cell lineages. Fluorescence microscopy analysis showed a unique pattern of expression of osteogenic, chondrogenic, muscle, and neural markers. The depicted "molecular signature" of these early uncommitted progenitors, in the absence of differentiation stimuli, is consistent with their multipotentiality and plasticity as suggested by several in vitro and in vivo studies.

Moviglia, G. A., et al. (2006). "Autoreactive T cells induce in vitro BM mesenchymal stem cell transdifferentiation to neural stem cells." Cytotherapy **8**(3): 196-201.

BACKGROUND: The degree of post-injury inflammation of the damaged area of a spinal cord is the main difference between the natural successful repair in inferior vertebrates and failure in superior vertebrates. The treatment of rats with anti-myelin lymphocytes after experimental spinal cord injury induces their functional recovery. On the other hand, mesenchymal stem cells (MSC) from adult BM implanted in injured areas recover the morphology and function of spinal cord in mammals. The purpose of this study was to determine whether there is a direct relationship between anti-nervous tissue T cells and MSC reparatory properties. METHODS: Circulating autoreactive lymphocytes of patients with spinal cord injuries and amyotrophic lateral sclerosis were isolated and activated in vitro. These cells were cocultured with autologous MSC for 2-15 days. Cocultures of non-selected lymphocytes were used as controls. RESULTS: After 48 h of coculture, MSC adopted a spindle shape with polarization of the cytoplasm that resembled bipolar neurons. Their nuclei diminished the nucleolus number and the chromatin lost its granular appearance. After 15 days of culture the cells developed the typical structure of a neural network. No morphologic changes were observed in control cultures. The differentiated cells reacted positively to tubuline III, GFAP and nestin. No differences were observed between the different patient cell sources. DISCUSSION: We observed that autoreactive cells may induce the transdifferentiation of MSC to neural stem cells. This T-cell-MSC interaction may be a common phenomenon during physiologic nerve tissue repair.

Niibe, K., et al. (2017). "The potential of enriched mesenchymal stem cells with neural crest cell phenotypes as a cell source for regenerative dentistry." Jpn Dent Sci Rev **53**(2): 25-33.

Effective regenerative treatments for periodontal tissue defects have recently been demonstrated using mesenchymal stromal/stem cells (MSCs). Furthermore, current bioengineering techniques have enabled de novo fabrication of tooth-perio dental units in mice. These cutting-edge technologies are expected to address unmet needs within regenerative dentistry. However, to achieve efficient and stable treatment outcomes, preparation of an appropriate stem cell source is essential. Many researchers are investigating the use of adult stem cells for regenerative dentistry; bone marrow-derived MSCs (BM-MSCs) are particularly promising and presently used clinically. However, current BM-MSC isolation techniques result in a heterogeneous, non-reproducible cell population because of a lack of identified distinct BM-MSC surface markers. Recently, specific subsets of cell surface markers for BM-MSCs have been reported in mice (PDGFRalpha(+) and Sca-1(+)) and humans (LNGFR(+), THY-1(+) and VCAM-1(+)), facilitating the isolation of unique enriched BM-MSCs (so-called "purified MSCs"). Notably, the enriched BM-MSC population contains neural crest-derived cells, which can differentiate into cells of neural crest- and mesenchymal lineages. In this review, characteristics of the enriched BM-MSCs are outlined with a focus on their potential application within future regenerative dentistry.

Oh, J. S., et al. (2010). "Hypoxia-preconditioned adipose tissue-derived mesenchymal stem cell increase the survival and gene expression of engineered neural stem cells in a spinal cord injury model." Neurosci Lett **472**(3): 215-219.

Hypoxic preconditioning (HP) is a novel strategy to make stem cells resistant to the ischemic environment they encounter after transplantation into injured tissue; this strategy improves survival of both the transplanted cells and the host cells at the injury site. Using both in vitro and in vivo injury models, we confirmed that HP-treated adipose tissue-derived mesenchymal stem cells (HP-AT-MSCs) increased cell survival and enhanced the expression of marker genes in DsRed-engineered neural stem cells (NSCs-DsRed). Similar to untreated AT-MSCs, HP-AT-MSCs had normal morphology and were positive for the cell surface markers CD90, CD105, and CD29, but not CD31. In three in vitro ischemic-mimicking injury models, HP-AT-MSCs significantly increased both the viability of NSCs-DsRed and the expression of DsRed and clearly reduced the number of annexin-V-positive apoptotic NSCs-DsRed and the expression of the apoptotic factor Bax. Consistent with the in vitro assay, co-transplantation of NSCs-DsRed with HP-AT-MSCs significantly improved the survival of the NSCs-DsRed, resulting in an increased expression of the DsRed reporter gene at the transplantation site in a rat spinal cord injury (SCI) model. These findings suggest that the co-transplantation of HP-AT-MSCs with engineered NSCs can improve both the cell survival and the gene expression of the engineered NSCs, indicating that this novel strategy can be used to augment the therapeutic efficacy of combined stem cell and gene therapies for SCI.

Ouchi, T., et al. (2016). "LNGFR(+)THY-1(+) human pluripotent stem cell-derived neural crest-like cells have the potential to develop into mesenchymal stem cells." Differentiation **92**(5): 270-280.

Mesenchymal stem cells (MSCs) are defined as non-hematopoietic, plastic-adherent, self-renewing cells that are capable of tri-lineage differentiation into bone, cartilage or fat in vitro. Thus, MSCs are promising candidates for cell-based medicine. However, classifications of MSCs have been defined retrospectively; moreover, this conventional criterion may be inaccurate due to contamination with other hematopoietic lineage cells. Human MSCs can be enriched by selection for LNGFR and THY-1, and this population may be analogous to murine PDGFRalpha(+)Sca-1(+) cells, which are developmentally derived from neural crest cells (NCCs). Murine NCCs were labeled by fluorescence, which provided definitive proof of neural crest lineage, however, technical considerations prevent the use of a similar approach to determine the origin of human LNGFR(+)THY-1(+) MSCs. To further clarify the origin of human MSCs, human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) were used in this study. Under culture conditions required for the induction of neural crest cells, human ESCs and iPSCs-derived cells highly expressed LNGFR and THY-1. These LNGFR(+)THY-1(+) neural crest-like cells, designated as LT-NCLCs, showed a strong potential to differentiate into both mesenchymal and neural crest lineages. LT-NCLCs proliferated to form colonies and actively migrated in response to serum concentration. Furthermore, we transplanted LT-NCLCs into chick embryos, and traced their potential for survival, migration and differentiation in the host environment. These results suggest that LNGFR(+)THY-1(+) cells identified following NCLC induction from ESCs/iPSCs shared similar potentials with multipotent MSCs.

Park, H. E., et al. (2011). "Real-time monitoring of neural differentiation of human mesenchymal stem

cells by electric cell-substrate impedance sensing." J Biomed Biotechnol **2011**: 485173.

Stem cells are useful for cell replacement therapy. Stem cell differentiation must be monitored thoroughly and precisely prior to transplantation. In this study we evaluated the usefulness of electric cell-substrate impedance sensing (ECIS) for in vitro real-time monitoring of neural differentiation of human mesenchymal stem cells (hMSCs). We cultured hMSCs in neural differentiation media (NDM) for 6 days and examined the time-course of impedance changes with an ECIS array. We also monitored the expression of markers for neural differentiation, total cell count, and cell cycle profiles. Cellular expression of neuron and oligodendrocyte markers increased. The resistance value of cells cultured in NDM was automatically measured in real-time and found to increase much more slowly over time compared to cells cultured in non-differentiation media. The relatively slow resistance changes observed in differentiating MSCs were determined to be due to their lower growth capacity achieved by induction of cell cycle arrest in G0/G1. Overall results suggest that the relatively slow change in resistance values measured by ECIS method can be used as a parameter for slowly growing neural-differentiating cells. However, to enhance the competence of ECIS for in vitro real-time monitoring of neural differentiation of MSCs, more elaborate studies are needed.

Qin, Y., et al. (2015). "Conversion of Adipose Tissue-Derived Mesenchymal Stem Cells to Neural Stem Cell-Like Cells by a Single Transcription Factor, Sox2." Cell Reprogram **17**(3): 221-226.

Adipose tissue is an attractive source of easily accessible adult candidate cells for regenerative medicine. Adipose tissue-derived mesenchymal stem cells (ADSCs) have multipotency and strong proliferation and differentiation capabilities in vitro. However, as mesodermal multipotent stem cells, whether the ADSCs can convert into induced neural stem cells (NSCs) has so far not been demonstrated. In this study, we found that normally the naive ADSCs cultured as either monolayer or spheres in NSC medium did not express Sox2 and Pax6 genes and proteins, and could not differentiate to neuron-like cells. However, when we introduced the Sox2 gene into ADSCs by retrovirus, they exhibited a typical NSC-like morphology, and could be passaged continuously, and expressed NSC specific markers Sox2 and Pax6. In addition, the ADSC-derived NSC-like cells displayed the ability to differentiate into neuron-like cells when switched to the differentiation culture medium, expressing neuronal markers, including Tuj1 and MAP2 genes and proteins. Our results suggest the ADSCs can be converted into induced NSC-like cells with a single transcription factor Sox2. This finding could provide another alternative cell source for cell therapy of neurological disorders.

Qiu, X. C., et al. (2015). "Donor mesenchymal stem cell-derived neural-like cells transdifferentiate into myelin-forming cells and promote axon regeneration in rat spinal cord transection." Stem Cell Res Ther **6**: 105.

INTRODUCTION: Severe spinal cord injury often causes temporary or permanent damages in strength, sensation, or autonomic functions below the site of the injury. So far, there is still no effective treatment for spinal cord injury. Mesenchymal stem cells (MSCs) have been used to repair injured spinal cord as an effective strategy. However, the low neural differentiation frequency of MSCs has limited its application. The present study attempted to explore whether the grafted MSC-derived neural-like cells in a gelatin sponge (GS) scaffold could maintain neural features or transdifferentiate into myelin-forming cells in the transected spinal cord. METHODS: We constructed an engineered tissue by co-seeding of MSCs with genetically enhanced expression of neurotrophin-3 (NT-3) and its high-affinity receptor tropomyosin receptor kinase C (TrkC) separately into a three-dimensional GS scaffold to promote the MSCs differentiating into neural-like cells and transplanted it into the gap of a completely transected rat spinal cord. The rats received extensive post-operation care, including cyclosporin A administrated once daily for 2 months. RESULTS: MSCs modified genetically could differentiate into neural-like cells in the MN + MT (NT-3-MSCs + TrKC-MSCs) group 14 days after culture in the GS scaffold. However, after the MSC-derived neural-like cells were transplanted into the injury site of spinal cord, some of them appeared to lose the neural phenotypes and instead transdifferentiated into myelin-forming cells at 8 weeks. In the latter, the MSC-derived myelin-forming cells established myelin sheaths associated with the host regenerating axons. And the injured host neurons were rescued, and axon regeneration was induced by grafted MSCs modified genetically. In addition, the cortical motor evoked potential and hindlimb locomotion were significantly ameliorated in the rat spinal cord transected in the MN + MT group compared with the GS and MSC groups. CONCLUSION: Grafted MSC-derived neural-like cells in the GS scaffold can transdifferentiate into

myelin-forming cells in the completely transected rat spinal cord.

Song, C., et al. (2015). "Use of Ferritin Expression, Regulated by Neural Cell-Specific Promoters in Human Adipose Tissue-Derived Mesenchymal Stem Cells, to Monitor Differentiation with Magnetic Resonance Imaging In Vitro." PLoS One **10**(7): e0132480.

The purpose of this study was to establish a method for monitoring the neural differentiation of stem cells using ferritin transgene expression, under the control of a neural-differentiation-inducible promoter, and magnetic resonance imaging (MRI). Human adipose tissue-derived mesenchymal stem cells (hADMSCs) were transduced with a lentivirus containing the human ferritin heavy chain 1 (FTH1) gene coupled to one of three neural cell-specific promoters: human synapsin 1 promoter (SYN1p, for neurons), human glial fibrillary acidic protein promoter (GFAPp, for astrocytes), and human myelin basic protein promoter (MBPp, for oligodendrocytes). Three groups of neural-differentiation-inducible ferritin-expressing (NDIFE) hADMSCs were established: SYN1p-FTH1, GFAPp-FTH1, and MBPp-FTH1. The proliferation rate of the NDIFE hADMSCs was evaluated using a Cell Counting Kit-8 assay. Ferritin expression was assessed with western blotting and immunofluorescent staining before and after the induction of differentiation in NDIFE hADMSCs. The intracellular iron content was measured with Prussian blue iron staining and inductively coupled plasma mass spectrometry. R2 relaxation rates were measured with MRI in vitro. The proliferation rates of control and NDIFE hADMSCs did not differ significantly ($P > 0.05$). SYN1p-FTH1, GFAPp-FTH1, and MBPp-FTH1 hADMSCs expressed specific markers of neurons, astrocytes, and oligodendrocytes, respectively, after neural differentiation. Neural differentiation increased ferritin expression twofold, the intracellular iron content threefold, and the R2 relaxation rate two- to threefold in NDIFE hADMSCs, resulting in notable hypointensity in T2-weighted images ($P < 0.05$). These results were cross-validated. Thus, a link between neural differentiation and MRI signals (R2 relaxation rate) was established in hADMSCs. The use of MRI and neural-differentiation-inducible ferritin expression is a viable method for monitoring the neural differentiation of hADMSCs.

Thrivikraman, G., et al. (2014). "Intermittent electrical stimuli for guidance of human mesenchymal stem cell lineage commitment towards neural-like cells on electroconductive substrates." Biomaterials **35**(24): 6219-6235.

In the context of the role of multiple physical factors in dictating stem cell fate, the present paper demonstrates the effectiveness of the intermittently delivered external electric field stimulation towards switching the stem cell fate to specific lineage, when cultured in the absence of biochemical growth factors. In particular, our findings present the ability of human mesenchymal stem cells (hMSCs) to respond to the electric stimuli by adopting extended neural-like morphology on conducting polymeric substrates. Polyaniline (PANI) is selected as the model system to demonstrate this effect, as the electrical conductivity of the polymeric substrates can be systematically tailored over a broad range (10(-9) to 10 S/cm) from highly insulating to conducting by doping with varying concentrations (10(-5) to 1 m) of HCl. On the basis of the culture protocol involving the systematic delivery of intermittent electric field (dc) stimulation, the parametric window of substrate conductivity and electric field strength was established to promote significant morphological extensions, with minimal cellular damage. A time dependent morphological change in hMSCs with significant filopodial elongation was observed after 7 days of electrically stimulated culture. Concomitant with morphological changes, a commensurate increase in the expression of neural lineage commitment markers such as nestin and betaIII tubulin was recorded from hMSCs grown on highly conducting substrates, as revealed from the mRNA expression analysis using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) as well as by immune-fluorescence imaging. Therefore, the present work establishes the key role of intermittent and systematic delivery of electric stimuli as guidance cues in promoting neural-like differentiation of hMSCs, when grown on electroconductive substrates.

Wang, X., et al. (2010). "Mouse mesenchymal stem cells can support human hematopoiesis both in vitro and in vivo: the crucial role of neural cell adhesion molecule." Haematologica **95**(6): 884-891.

BACKGROUND: We previously established a mesenchymal stem cell line (FMS/PA6-P) from the bone marrow adherent cells of fetal mice. The cell line expresses a higher level of neural cell adhesion molecule and shows greater hematopoiesis-supporting capacity in mice than other murine stromal cell lines. DESIGN AND METHODS: Since there is 94% homology between human and murine neural cell adhesion molecule, we examined whether FMS/PA6-P cells support human hematopoiesis and

whether neural cell adhesion molecules expressed on FMS/PA6-P cells contribute greatly to the human hematopoiesis-supporting ability of the cell line. RESULTS: When lineage-negative cord blood mononuclear cells were co-cultured on the FMS/PA6-P cells, a significantly greater hematopoietic stem cell-enriched population (CD34(+)CD38(-) cells) was obtained than in the culture without the FMS/PA6-P cells. Moreover, when lineage-negative cord blood mononuclear cells were cultured on FMS/PA6-P cells and transplanted into SCID mice, a significantly larger proportion of human CD45(+) cells and CD34(+)CD38(-) cells were detected in the bone marrow of SCID mice than in the bone marrow of SCID mice that had received lineage-negative cord blood mononuclear cells cultured without FMS/PA6-P cells. Furthermore, we found that direct cell-to-cell contact between the lineage-negative cord blood mononuclear cells and the FMS/PA6-P cells was essential for the maximum expansion of the mononuclear cells. The addition of anti-mouse neural cell adhesion molecule antibody to the culture significantly inhibited their contact and the proliferation of lineage-negative cord blood mononuclear cells. CONCLUSIONS: These findings suggest that neural cell adhesion molecules expressed on FMS/PA6-P cells play a crucial role in the human hematopoiesis-supporting ability of the cell line.

Xu, X., et al. (2014). "Neural differentiation of mesenchymal stem cells influences their chemotactic responses to stromal cell-derived factor-1alpha." Cell Mol Neurobiol **34**(7): 1047-1058.

Mesenchymal stem cells (MSCs) are proposed as a promising source for cell-based therapies in neural disease. Although increasing numbers of studies have been devoted to the delineation of factors involved in the migration of MSCs, the relationship between the chemotactic response and the differentiation status of these cells is still unclear. In the present study, we demonstrated that MSCs in varying neural differentiation states display various chemotactic responses to stromal cell-derived factor-1alpha (SDF-1alpha). The chemotactic responses of MSCs under different differentiation stages in response to SDF-1alpha were analyzed by Boyden chamber, and the results showed that cells of undifferentiation, 24-h preinduction, 5-h induction, and 18-h maintenance states displayed a stronger chemotactic response to SDF-1alpha, while 48-h maintenance did not. Further, we found that the phosphorylation levels of PI3K/Akt, ERK1/2, SAPK/JNK, and p38MAPK are closely related to the differentiation states of MSCs subjected to SDF-1alpha, and finally, inhibition of SAPK/JNK signaling significantly attenuates SDF-1alpha-stimulated transfilter migration of MSCs of undifferentiation, 24-h preinduction, 18-h maintenance, and 48-h maintenance, but not MSCs of 5-h induction. Meanwhile, interference with PI3K/Akt, p38MAPK, or ERK1/2 signaling prevents only cells at certain differentiation state from migrating in response to SDF-1alpha. Collectively, these results demonstrate that MSCs in varying neural differentiation states have different migratory capacities, thereby illuminating optimization of the therapeutic potential of MSCs to be used for neural regeneration after injury.

Yang, H. J., et al. (2011). "A novel role for neural cell adhesion molecule in modulating insulin signaling and adipocyte differentiation of mouse mesenchymal stem cells." J Cell Sci **124**(Pt 15): 2552-2560.

Neural cell adhesion molecule (NCAM) has recently been found on adult stem cells, but its biological significance remains largely unknown. In this study, we used bone-marrow-derived mesenchymal stem cells (MSCs) from wild-type and NCAM knockout mice to investigate the role of NCAM in adipocyte differentiation. It was demonstrated that NCAM isoforms 180 and 140 but not NCAM-120 are expressed on almost all wild-type MSCs. Upon adipogenic induction, Ncam(-/-) MSCs exhibited a marked decrease in adipocyte differentiation compared with wild-type cells. The role of NCAM in adipocyte differentiation was also confirmed in NCAM-silenced preadipocyte 3T3-L1 cells, which also had a phenotype with reduced adipogenic potential. In addition, we found that Ncam(-/-) MSCs appeared to be insulin resistant, as shown by their impaired insulin signaling cascade, such as the activation of the insulin-IGF-1 receptor, PI3K-Akt and CREB pathways. The PI3K-Akt inhibitor, LY294002, completely blocked adipocyte differentiation of MSCs, unveiling that the reduced adipogenic potential of Ncam(-/-) MSCs is due to insulin resistance as a result of loss of NCAM function. Furthermore, insulin resistance of Ncam(-/-) MSCs was shown to be associated with induction of tumor necrosis factor alpha (TNF-alpha), a key mediator of insulin resistance. Finally, we demonstrated that re-expression of NCAM-180, but not NCAM-140, inhibits induction of TNF-alpha and thereby improves insulin resistance and adipogenic potential of Ncam(-/-) MSCs. Our results suggest a novel role of NCAM in promoting insulin signaling and adipocyte differentiation of adult stem cells. These findings raise the possibility of using NCAM intervention to improve insulin resistance.

Zeng, X., et al. (2015). "Integration of donor mesenchymal stem cell-derived neuron-like cells into host neural network after rat spinal cord transection." Biomaterials **53**: 184-201.

Functional deficits following spinal cord injury (SCI) primarily attribute to loss of neural connectivity. We therefore tested if novel tissue engineering approaches could enable neural network repair that facilitates functional recovery after spinal cord transection (SCT). Rat bone marrow-derived mesenchymal stem cells (MSCs), genetically engineered to overexpress TrkC, receptor of neurotrophin-3 (NT-3), were pre-differentiated into cells carrying neuronal features via co-culture with NT-3 overproducing Schwann cells in 3-dimensional gelatin sponge (GS) scaffold for 14 days in vitro. Intra-GS formation of MSC assemblies emulating neural network (MSC-GS) were verified morphologically via electron microscopy (EM) and functionally by whole-cell patch clamp recording of spontaneous post-synaptic currents. The differentiated MSCs still partially maintained prototypic property with the expression of some mesodermal cytokines. MSC-GS or GS was then grafted acutely into a 2 mm-wide transection gap in the T9-T10 spinal cord segments of adult rats. Eight weeks later, hindlimb function of the MSC-GS-treated SCT rats was significantly improved relative to controls receiving the GS or lesion only as indicated by BBB score. The MSC-GS transplantation also significantly recovered cortical motor evoked potential (CMEP). Histologically, MSC-derived neuron-like cells maintained their synapse-like structures in vivo; they additionally formed similar connections with host neurites (i.e., mostly serotonergic fibers plus a few corticospinal axons; validated by double-labeled immuno-EM). Moreover, motor cortex electrical stimulation triggered c-fos expression in the grafted and lumbar spinal cord cells of the treated rats only. Our data suggest that MSC-derived neuron-like cells resulting from NT-3-TrkC-induced differentiation can partially integrate into transected spinal cord and this strategy should be further investigated for reconstructing disrupted neural circuits.

Zhou, J., et al. (2012). "Neural cell injury microenvironment induces neural differentiation of human umbilical cord mesenchymal stem cells." Neural Regen Res **7**(34): 2689-2697.

This study aimed to investigate the neural differentiation of human umbilical cord mesenchymal stem cells (hUCMSCs) under the induction of injured neural cells. After in vitro isolation and culture, passage 5 hUCMSCs were used for experimentation. hUCMSCs were co-cultured with normal or Abeta1-40-injured PC12 cells, PC12 cell supernatant or PC12 cell lysate in a Transwell co-culture system. Western blot analysis and flow cytometry results showed that choline acetyltransferase and microtubule-associated protein 2, a specific marker for neural cells, were expressed in hUCMSCs under various culture conditions, and highest expression was observed in the hUCMSCs co-cultured with injured PC12 cells. Choline acetyltransferase and microtubule-associated protein 2 were not expressed in hUCMSCs cultured alone (no treatment). Cell Counting Kit-8 assay results showed that hUCMSCs under co-culture conditions promoted the proliferation of injured PC12 cells. These findings suggest that the microenvironment during neural tissue injury can effectively induce neural cell differentiation of hUCMSCs. These differentiated hUCMSCs likely accelerate the repair of injured neural cells.

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