Neural Stem Cell Research Literatures

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Abstract: 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.

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Key words: stem cell; neural; life; research; literature

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.

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

Acharya, M. M., S. Rosi, et al. "Human neural stem cell transplantation provides long-term restoration of neuronal plasticity in the irradiated hippocampus." Cell Transplant. 2015;24(4):691-702. doi: 10.3727/096368914X684600. Epub 2014 Oct 6.

For the majority of CNS malignancies, radiotherapy provides the best option for forestalling tumor growth, but is frequently associated with debilitating and progressive cognitive dysfunction. Despite the recognition of this serious side effect, satisfactory long-term solutions are not currently available and have prompted our efforts to explore the potential therapeutic efficacy of cranial stem cell transplants. We have demonstrated that intrahippocampal transplantation of human neural stem cells (hNSCs) can provide long-lasting cognitive benefits using an athymic rat model subjected to cranial irradiation. To explore the possible mechanisms underlying the capability of engrafted cells to ameliorate radiation-induced cognitive dysfunction we analyzed the expression patterns of the behaviorally induced activity-regulated cytoskeleton-associated protein (Arc) in the hippocampus at 1 and 8 months postgrafting. While immunohistochemical analyses revealed a small fraction (4.5%) of surviving hNSCs in the irradiated brain that did not express neuronal or astroglial makers, hNSC transplantation impacted the irradiated microenvironment of the host brain by promoting the expression of Arc at both time points. Arc is known to play key roles in the neuronal mechanisms underlying long-term synaptic plasticity and memory and provides a reliable marker for detecting neurons that are actively engaged in spatial and contextual information processing associated with memory consolidation. Cranial irradiation significantly reduced the number of pyramidal (CA1) and granule neurons (DG) expressing behaviorally induced Arc at 1 and 8 months postirradiation. Transplantation of hNSCs restored the expression of plasticity-related Arc in the host brain to control levels. These findings suggest that hNSC transplantation promotes the long-term recovery of host hippocampal neurons and indicates that one mechanism promoting the preservation of cognition after irradiation involves trophic support from engrafted cells.

Arulmoli, J., M. M. Pathak, et al. "Static stretch affects neural stem cell differentiation in an extracellular matrix-dependent manner." Sci Rep. 2015 Feb 17;5:8499. doi: 10.1038/srep08499.

Neural stem and progenitor cell (NSPC) fate is strongly influenced by mechanotransduction as modulation of substrate stiffness affects lineage choice. Other types of mechanical stimuli, such as stretch (tensile strain), occur during CNS development and trauma, but their consequences for NSPC differentiation have not been reported. We delivered a 10% static equibiaxial stretch to NSPCs and examined effects on differentiation. We found static stretch specifically impacts NSPC differentiation into oligodendrocytes, but not neurons or astrocytes, and

this effect is dependent on particular extracellular matrix (ECM)-integrin linkages. Generation of oligodendrocytes from NSPCs was reduced on laminin, an outcome likely mediated by the alpha6 laminin-binding integrin, whereas similar effects were not observed for NSPCs on fibronectin. Our data demonstrate a direct role for tensile strain in dictating the lineage choice of NSPCs and indicate the dependence of this phenomenon on specific substrate materials, which should be taken into account for the design of biomaterials for NSPC transplantation.

Arya, R., T. Sarkissian, et al. "Neural stem cell progeny regulate stem cell death in a Notch and Hox dependent manner." Cell Death Differ. 2015 Jan 30. doi: 10.1038/cdd.2014.235.

Cell death is a prevalent, well-controlled and fundamental aspect of development, particularly in the nervous system. In Drosophila, specific neural stem cells are eliminated by apoptosis during embryogenesis. In the absence of apoptosis, these stem cells continue to divide, resulting in a dramatically hyperplastic central nervous system and adult lethality. Although core cell death pathways have been well described, the spatial, temporal and cell identity cues that activate the cell death machinery in specific cells are largely unknown. We identified a cis-regulatory region that controls the transcription of the cell death activators reaper, grim and sickle exclusively in neural stem cells. Using a reporter generated from this regulatory region, we found that Notch activity is required for neural stem cell death. Notch regulates the expression of the abdominal A homeobox protein, which provides important spatial cues for death. Importantly, we show that pro-apoptotic Notch signaling is activated by the Delta ligand expressed on the neighboring progeny of the stem cell. Thus we identify a previously undescribed role for progeny in regulating the proper developmental death of their parental stem cells. Cell Death and Differentiation advance online publication, 30 January 2015; doi:10.1038/cdd.2014.235.

Bauer, R., M. Kaiser, et al. "A computational model incorporating neural stem cell dynamics reproduces glioma incidence across the lifespan in the human population." PLoS One. 2014 Nov 19;9(11):e111219. doi: 10.1371/journal.pone.0111219. eCollection 2014.

Glioma is the most common form of primary brain tumor. Demographically, the risk of occurrence increases until old age. Here we present a novel computational model to reproduce the probability of glioma incidence across the lifespan. Previous mathematical models explaining glioma incidence are framed in a rather abstract way, and do not directly relate to empirical findings. To decrease this gap between theory and experimental observations, we incorporate recent data on cellular and molecular factors underlying gliomagenesis. Since evidence implicates the adult neural stem cell as the likely cell-of-origin of glioma, we have incorporated empirically-determined estimates of neural stem cell number, cell division rate, mutation rate and oncogenic potential into our model. We demonstrate that our model yields results which match actual demographic data in the human population. In particular, this model accounts for the observed peak incidence of glioma at approximately 80 years of age, without the need to assert differential susceptibility throughout the population. Overall, our model supports the hypothesis that glioma is caused by randomly-occurring oncogenic mutations within the neural stem cell population. Based on this model, we assess the influence of the (experimentally indicated) decrease in the number of neural stem cells and increase of cell division rate during aging. Our model provides multiple testable predictions, and suggests that different temporal sequences of oncogenic mutations can lead to tumorigenesis. Finally, we conclude that four or five oncogenic mutations are sufficient for the formation of glioma.

Bender, R. H., K. M. Haigis, et al. "Activated k-ras, but not h-ras or N-ras, regulates brain neural stem cell proliferation in a raf/rb-dependent manner." Stem Cells. 2015 Jun;33(6):1998-2010. doi: 10.1002/stem.1990.

Neural stem cells (NSCs) give rise to all the major cell types in the brain, including neurons, oligodendrocytes, and astrocytes. However, the intracellular signaling pathways that govern brain NSC proliferation and differentiation have been incompletely characterized to date. Since some neurodevelopmental brain disorders (Costello syndrome and Noonan syndrome) are caused by germline activating mutations in the RAS genes, Ras small GTPases are likely critical regulators of brain NSC function. In the mammalian brain, Ras exists as three distinct molecules (H-Ras, K-Ras, and N-Ras), each with different subcellular localizations, downstream signaling effectors, and biological effects. Leveraging a novel series of conditional-activated Ras molecule-expressing genetically engineered mouse strains, we demonstrate that activated K-Ras, but not H-Ras or N-Ras, expression increases brain NSC growth in a Raf-dependent, but Mek-independent, manner. Moreover, we show that activated K-Ras regulation of brain NSC proliferation requires Raf binding and suppression of retinoblastoma (Rb) function. Collectively, these observations establish tissue-specific differences in activated Ras molecule regulation of brain cell growth that operate through a

noncanonical mechanism. Stem Cells 2015;33:1998-2010.

Berrios, V. O., N. M. Boukli, et al. "Paraoxon and Pyridostigmine Interfere with Neural Stem Cell Differentiation." Neurochem Res. 2015 Mar 11.

Acetylcholinesterase (AChE) inhibition has been described as the main mechanism of organophosphate (OP)-evoked toxicity. OPs represent a human health threat, because chronic exposure to low doses can damage the developing brain, and acute exposure can produce long-lasting damage to adult brains, despite post-exposure medical countermeasures. Although the main mechanism of OP toxicity is AChE inhibition, several lines of evidence suggest that OPs also act by other mechanisms. We hypothesized that rat neural progenitor cells extracted on embryonic day 14.5 would be affected by constant inhibition of AChE from chronic exposure to OP or pyridostigmine (a reversible AChE blocker) during differentiation. In this work, the OP paraoxon decreased cell viability in concentrations >50 muM, as measured with the MTT assay; however, this effect was not dose-dependent. Reduced viability could not be attributed to blockade of AChE activity, since treatment with 200 microM pyridostigmine did not affect cell viability, even after 6 days. Although changes in protein expression patterns were noted in both treatments, the distribution of differentiated phenotypes, such as the percentages of neurons and glial cells, was not altered, as determined by flow cytometry. Since paraoxon and pyridostigmine each decreased neurite outgrowth (but did not prevent differentiation), we infer that developmental patterns may have been affected.

Binder, E., D. Natarajan, et al. "Enteric neurospheres are not specific to neural crest cultures: implications for neural stem cell therapies." PLoS One. 2015 Mar 23;10(3):e0119467. doi: 10.1371/journal.pone.0119467. eCollection 2015.

OBJECTIVES: Enteric neural stem cells provide hope of curative treatment for enteric neuropathies. Current protocols for their harvesting from humans focus on the generation of 'neurospheres' from cultures of dissociated gut tissue. The study aims to better understand the derivation, generation and composition of enteric neurospheres. DESIGN: Gut tissue was obtained from Wnt1-Cre;Rosa26Yfp/Yfp transgenic mice (constitutively labeled neural crest cells) and paediatric patients. Gut cells were cultured either unsorted (mixed neural crest/non-neural crest), or following FACS selection into neural crest (murine-YFP+ve/human-p75+ve) or non-neural crest (YFP-ve/p75-ve) populations. Cultures and resultant neurospheres were characterized using immunolabelling in vitro and following transplantation in vivo. RESULTS: Cultures of (i) unsorted, (ii) neural crest, and (iii) non-neural crest cell populations generated neurospheres similar in numbers, size and morphology. Unsorted neurospheres were highly heterogeneous for neural crest content. Neural crest-derived (YFP+ve/p75+ve) neurospheres contained only neural derivatives (neurons and glia) and were devoid of non-neural cells (i.e. negative for SMA, c-Kit), with the converse true for non-neural crest-derived (YFP-ve/p75-ve) 'neurospheres'. Under differentiation conditions only YFP+ve cells gave rise to neural derivatives. Both YFP+ve and YFP-ve cells displayed proliferation and spread upon transplantation in vivo, but YFP-ve cells did not locate or integrate within the host ENS. CONCLUSIONS: Spherical accumulations of cells, so-called 'neurospheres' forming in cultures of dissociated gut contain variable proportions of neural crest-derived cells. If they are to be used for ENS cell replacement therapy then improved protocols for their generation, including cell selection, should be sought in order to avoid inadvertent transplantation of non-therapeutic, non-ENS cells.

Castelo-Branco, G., T. Lilja, et al. "Neural stem cell differentiation is dictated by distinct actions of nuclear receptor corepressors and histone deacetylases." Stem Cell Reports. 2014 Sep 9;3(3):502-15. doi: 10.1016/j.stemcr.2014.07.008. Epub 2014 Aug 28.

Signaling factors including retinoic acid (RA) and thyroid hormone (T3) promote neuronal, oligodendrocyte, and astrocyte differentiation of cortical neural stem cells (NSCs). However, the functional specificity of transcriptional repressor checkpoints controlling these differentiation programs remains unclear. Here, we show by genome-wide analysis that histone deacetylase (HDAC)2 and HDAC3 show overlapping and distinct promoter occupancy at neuronal and oligodendrocyte-related genes in NSCs. The absence of HDAC3, but not HDAC2, initiated a neuronal differentiation pathway in NSCs. The ablation of the corepressor NCOR or HDAC2, in conjunction with T3 treatment, resulted in increased expression of oligodendrocyte genes, revealing a direct HDAC2-mediated repression of Sox8 and Sox10 expression. Interestingly, Sox10 was required also for maintaining the more differentiated state by repression of stem cell programming factors such as Sox2 and Sox9. Distinct and nonredundant actions of NCORs and HDACs are thus critical for control of lineage progression and differentiation programs in neural progenitors.

Cerbini, T., R. Funahashi, et al. "Transcription activator-like effector nuclease (TALEN)-mediated

CLYBL targeting enables enhanced transgene expression and one-step generation of dual reporter human induced pluripotent stem cell (iPSC) and neural stem cell (NSC) lines." PLoS One. 2015 Jan 14;10(1):e0116032. doi: 10.1371/journal.pone.0116032. eCollection 2015.

Targeted genome engineering to robustly express transgenes is an essential methodology for stem cell-based research and therapy. Although designer nucleases have been used to drastically enhance gene editing efficiency, targeted addition and stable expression of transgenes to date is limited at single gene/locus and mostly PPP1R12C/AAVS1 in human stem cells. Here we constructed transcription activator-like effector nucleases (TALENs) targeting the safe-harbor like gene CLYBL to mediate reporter gene integration at 38%-58% efficiency, and used both AAVS1-TALENs and CLYBL-TALENs to simultaneously knock-in multiple reporter genes at dual safe-harbor loci in human induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs). The CLYBL-TALEN engineered cell lines maintained robust reporter expression during self-renewal and differentiation, and revealed that CLYBL targeting resulted in stronger transgene expression and less perturbation on local gene expression than PPP1R12C/AAVS1. TALEN-mediated CLYBL engineering provides improved transgene expression and options for multiple genetic modification in human stem cells.

Edri, R., Y. Yaffe, et al. "Analysing human neural stem cell ontogeny by consecutive isolation of Notch active neural progenitors." Nat Commun. 2015 Mar 23;6:6500. doi: 10.1038/ncomms7500.

Decoding heterogeneity of pluripotent stem cell (PSC)-derived neural progeny is fundamental for revealing the origin of diverse progenitors, for defining their lineages, and for identifying fate determinants driving transition through distinct potencies. Here we have prospectively isolated consecutively appearing PSC-derived primary progenitors based on their Notch activation state. We first isolate early neuroepithelial cells and show their broad Notch-dependent developmental and proliferative potential. Neuroepithelial cells further yield successive Notch-dependent functional primary progenitors, from early and midneurogenic radial glia and their derived basal progenitors, to gliogenic radial glia and adult-like neural progenitors, together recapitulating hallmarks of neural stem cell (NSC) ontogeny. Gene expression profiling reveals dynamic stage-specific transcriptional patterns that may link development of distinct progenitor identities through Notch activation. Our observations provide a platform for characterization and manipulation of distinct progenitor cell types amenable for developing streamlined neural lineage specification paradigms for modelling development in health and disease.

Ferent, J., L. Cochard, et al. "Genetic activation of Hedgehog signaling unbalances the rate of neural stem cell renewal by increasing symmetric divisions." Stem Cell Reports. 2014 Aug 12;3(2):312-23. doi: 10.1016/j.stemcr.2014.05.016. Epub 2014 Jun 19.

In the adult brain, self-renewal is essential for the persistence of neural stem cells (NSCs) throughout life, but its regulation is still poorly understood. One NSC can give birth to two NSCs or one NSC and one transient progenitor. A correct balance is necessary for the maintenance of germinal areas, and understanding the molecular mechanisms underlying NSC division mode is clearly important. Here, we report a function of the Sonic Hedgehog (SHH) receptor Patched in the direct control of long-term NSC self-renewal in the subependymal zone. We show that genetic conditional activation of SHH signaling in adult NSCs leads to their expansion and the depletion of their direct progeny. These phenotypes are associated in vitro with an increase in NSC symmetric division in a process involving NOTCH signaling. Together, our results demonstrate a tight control of adult neurogenesis and NSC renewal driven by Patched.

Fischer, B., K. Azim, et al. "E-proteins orchestrate the progression of neural stem cell differentiation in the postnatal forebrain." Neural Dev. 2014 Oct 29;9(1):23. doi: 10.1186/1749-8104-9-23.

BACKGROUND: Neural stem cell (NSC) differentiation is a complex multistep process that persists in specific regions of the postnatal forebrain and requires tight regulation throughout life. The transcriptional control of NSC proliferation and specification involves Class II (proneural) and Class V (Id1-4) basic helix-loop-helix (bHLH) proteins. In this study, we analyzed the pattern of expression of their dimerization partners, Class I bHLH proteins (E-proteins), and explored their putative role in orchestrating postnatal subventricular zone (SVZ) neurogenesis. RESULTS: Overexpression of a dominant-negative form of the E-protein E47 (dnE47) confirmed a crucial role for bHLH transcriptional networks in postnatal neurogenesis by dramatically blocking SVZ NSC differentiation. In situ hybridization was used in combination with RT-qPCR to measure and compare the level of expression of E-protein transcripts (E2-2, E2A, and HEB) in the neonatal and adult SVZ as well as in magnetic affinity cell sorted progenitor cells and neuroblasts. Our results evidence that E-protein transcripts, in particular E2-2 and E2A, are enriched in the postnatal SVZ with expression levels increasing as cells engage

towards neuronal differentiation. To investigate the role of E-proteins in orchestrating lineage progression, both in vitro and in vivo gain-of-function and loss-of-function experiments were performed for individual E-proteins. Overexpression of E2-2 and E2A promoted SVZ neurogenesis by enhancing not only radial glial cell differentiation but also cell cycle exit of their progeny. Conversely, knock-down by shRNA electroporation resulted in opposite effects. Manipulation of E-proteins and/or Ascl1 in SVZ NSC cultures indicated that those effects were Ascl1 dependent, although they could not solely be attributed to an Ascl1-induced switch from promoting cell proliferation to triggering cell cycle arrest and differentiation. CONCLUSIONS: In contrast to former concepts, suggesting ubiquitous expression and subsidiary function for E-proteins to foster postnatal neurogenesis, this work unveils E-proteins as being active players in the orchestration of postnatal SVZ neurogenesis.

Flachsbarth, K., K. Kruszewski, et al. "Neural stem cell-based intraocular administration of ciliary neurotrophic factor attenuates the loss of axotomized ganglion cells in adult mice." Invest Ophthalmol Vis Sci. 2014 Sep 30;55(11):7029-39. doi: 10.1167/iovs.14-15266.

PURPOSE: To analyze the neuroprotective effect of intravitreally grafted neural stem (NS) cells genetically modified to secrete ciliary neurotrophic factor (CNTF) on intraorbitally lesioned retinal ganglion cells (RGCs) in adult mice. METHODS: Adherently cultivated NS cells were genetically modified to express a secretable variant of mouse CNTF together with the fluorescent reporter protein Venus. Clonal CNTF-secreting NS cell lines were established using fluorescence activated cell sorting, and intravitreally grafted into adult mice 1 day after an intraorbital crush of the optic nerve. Brn-3a-positive RGCs were counted in flat-mounted retinas at different postlesion intervals to evaluate the neuroprotective effect of the CNTF-secreting NS cells on the axotomized RGCs. Anterograde axonal tracing experiments were performed to analyze the regrowth of the injured RGC axons in CNTF-treated retinas. RESULTS: Intravitreally grafted NS cells preferentially differentiated into astrocytes that survived in the host eyes, stably expressed CNTF, and significantly attenuated the loss of the axotomized RGCs over a period of at least 4 months, the latest postlesion time point analyzed. Depending on the postlesion interval analyzed, the number of RGCs in eyes with grafted CNTF-secreting NS cells was 2.8-fold to 6.4-fold higher than in eyes with grafted control NS cells. The CNTF-secreting NS cells additionally induced long-distance regrowth of the lesioned RGC axons. CONCLUSIONS: Genetically modified clonal NS cell lines may serve as a useful tool for preclinical studies aimed at evaluating the therapeutic potential of a sustained cell-based intravitreal administration of neuroprotective factors in mouse models of glaucoma.

Ginisty, A., A. Gely-Pernot, et al. "Evidence for a subventricular zone neural stem cell phagocytic activity stimulated by the vitamin K-dependent factor protein S." Stem Cells. 2015 Feb;33(2):515-25. doi: 10.1002/stem.1862.

Neural stem cells, whose major reservoir in the adult mammalian brain is the subventricular zone (SVZ), ensure neuropoiesis, a process during which many generated cells die. Removal of dead cells and debris by phagocytes is necessary for tissue homeostasis. Using confocal and electron microscopy, we demonstrate that cultured SVZ cells phagocytose both 1 and 2 microm latex beads and apoptotic cell-derived fragments. We determine by flow cytometry that phagocytic cells represent more than 10% of SVZ cultured cells. Phenotyping of SVZ cells using nestin, GFAP, Sox2, or LeX/SSEA and quantification of aldehyde dehydrogenase (ALDH) activity, reveals that cells with neural stem-cell features phagocytose and represent more than 30% of SVZ phagocytic cells. In vivo, nestin-, Sox2-, and ALDH-expressing neural stem-like cells engulfed latex beads or apoptotic cell-derived fragments that were injected into mice lateral brain ventricles. We show also that SVZ cell phagocytic activity is an active process, which depends both on cytoskeleton dynamic and on recognition of phosphatidylserine eat-me signal, and is stimulated by the vitamin K-dependent factor protein S (ProS). ProS neutralizing antibodies inhibit SVZ cell phagocytic activity and exposure of SVZ cells to apoptotic cell-derived fragments induces a transient Mer tyrosine kinase receptor (MerTK) phosphorylation. Conversely, MerTK blocking antibodies impair both basal and ProS-stimulated SVZ cell phagocytic activity. By revealing that neural stem-like cells act within the SVZ neurogenic niche as phagocytes and that the ProS/MerTK path represents an endogenous regulatory mechanism for SVZ cell phagocytic activity, the present report opens-up new perspectives for both stem cell biology and brain physiopathology.

Gondi, V., S. L. Pugh, et al. "Preservation of memory with conformal avoidance of the hippocampal neural stem-cell compartment during whole-brain radiotherapy for brain metastases (RTOG 0933): a phase II multi-institutional trial." J Clin Oncol. 2014 Dec 1;32(34):3810-6. doi: 10.1200/JCO.2014.57.2909. Epub 2014 Oct 27.

PURPOSE: Hippocampal neural stem-cell injury during whole-brain radiotherapy (WBRT) may play a role in memory decline. Intensity-modulated radiotherapy can be used to avoid conformally the hippocampal neural stem-cell compartment during WBRT (HA-WBRT). RTOG 0933 was a single-arm phase II study of HA-WBRT for brain metastases with prespecified comparison with a historical control of patients treated with WBRT without hippocampal avoidance. PATIENTS AND METHODS: Eligible adult patients with brain metastases received HA-WBRT to 30 Gy in 10 fractions. Standardized cognitive function and quality-of-life (QOL) assessments were performed at baseline and 2, 4, and 6 months. The primary end point was the Hopkins Verbal Learning Test-Revised Delayed Recall (HVLT-R DR) at 4 months. The historical control demonstrated a 30% mean relative decline in HVLT-R DR from baseline to 4 months. To detect a mean relative decline </= 15% in HVLT-R DR after HA-WBRT, 51 analyzable patients were required to ensure 80% statistical power with alpha = 0.05. RESULTS: Of 113 patients accrued from March 2011 through November 2012, 42 patients were analyzable at 4 months. Mean relative decline in HVLT-R DR from baseline to 4 months was 7.0% (95% CI, -4.7% to 18.7%), significantly lower in comparison with the historical control (P < .001). No decline in QOL scores was observed. Two grade 3 toxicities and no grade 4 to 5 toxicities were reported. Median survival was 6.8 months. CONCLUSION: Conformal avoidance of the hippocampus during WBRT is associated with preservation of memory and QOL as compared with historical series.

Gregoire, C. A., B. L. Goldenstein, et al. "Endogenous neural stem cell responses to stroke and spinal cord injury." Glia. 2015 Aug;63(8):1469-82. doi: 10.1002/glia.22851. Epub 2015 Apr 29.

Stroke and spinal cord injury (SCI) are among the most frequent causes of central nervous system (CNS) dysfunction, affecting millions of people worldwide each year. The personal and financial costs for affected individuals, their families, and the broader communities are enormous. Although the mammalian CNS exhibits little spontaneous regeneration and self-repair, recent discoveries have revealed that subpopulations of glial cells in the adult forebrain subventricular zone and the spinal cord ependymal zone possess neural stem cell properties. These endogenous neural stem cells react to stroke and SCI by contributing a significant number of new neural cells to formation of the glial scar. These findings have raised hopes that new therapeutic strategies can be designed based on appropriate modulation of endogenous neural stem cell responses to CNS injury. Here, we review the responses of forebrain and spinal cord neural stem cells to stroke and SCI, the role of these responses in restricting injury-induced tissue loss, and the possibility of directing these responses to promote anatomical and functional repair of the CNS. GLIA 2015;63:1469-1482.

Hillje, A. L., E. Beckmann, et al. "The neural stem cell fate determinant TRIM32 regulates complex behavioral traits." Front Cell Neurosci. 2015 Mar 18;9:75. doi: 10.3389/fncel.2015.00075. eCollection 2015.

In mammals, new neurons are generated throughout the entire lifespan in two restricted areas of the brain, the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ)-olfactory bulb (OB) system. In both regions newborn neurons display unique properties that clearly distinguish them from mature neurons. Enhanced excitability and increased synaptic plasticity enables them to add specific properties to information processing by modulating the existing local circuitry of already established mature neurons. Hippocampal neurogenesis has been suggested to play a role in spatial-navigation learning, spatial memory, and spatial pattern separation. Cumulative evidences implicate that adult-born OB neurons contribute to learning processes and odor memory. We recently demonstrated that the cell fate determinant TRIM32 is upregulated in differentiating neuroblasts of the SVZ-OB system in the adult mouse brain. The absence of TRIM32 leads to increased progenitor cell proliferation and less cell death. Both effects accumulate in an overproduction of adult-generated OB neurons. Here, we present novel data from behavioral studies showing that such an enhancement of OB neurogenesis not necessarily leads to increased olfactory performance but in contrast even results in impaired olfactory capabilities. In addition, we show at the cellular level that TRIM32 protein levels increase during differentiation of neural stem cells (NSCs). At the molecular level, several metabolic intermediates that are connected to glycolysis, glycine, or cysteine metabolism are deregulated in TRIM32 knockout mice brain tissue. These metabolomics pathways are directly or indirectly linked to anxiety or depression like behavior. In summary, our study provides comprehensive data on how the impairment of neurogenesis caused by the loss of the cell fate determinant TRIM32 causes a decrease of olfactory performance as well as a deregulation of metabolomic pathways that are linked to mood disorders.

Itakura, G., Y. Kobayashi, et al. "Controlling immune rejection is a fail-safe system against potential

tumorigenicity after human iPSC-derived neural stem cell transplantation." PLoS One. 2015 Feb 23;10(2):e0116413. doi: 10.1371/journal.pone.0116413. eCollection 2015.

Our previous work reported functional recovery after transplantation of mouse and human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) into rodent models of spinal cord injury (SCI). Although hiPSC-NS/PCs proved useful for the treatment of SCI, the tumorigenicity of the transplanted cells must be resolved before they can be used in clinical applications. The current study sought to determine the feasibility of ablation of the tumors formed after hiPSC-NS/PC transplantation through immunoregulation. Tumorigenic hiPSC-NS/PCs were transplanted into the intact spinal cords of immunocompetent BALB/cA mice with or without immunosuppressant treatment. In vivo bioluminescence imaging was used to evaluate the chronological survival and growth of the transplanted cells. The graft survival rate was 0% in the group without immunosuppressants versus 100% in the group with immunosuppressants. Most of the mice that received immunosuppressants exhibited hind-limb paralysis owing to tumor growth at 3 months after iPSC-NS/PC transplantation. Histological analysis showed that the tumors shared certain characteristics with low-grade gliomas rather than with teratomas. After confirming the progression of the tumors in immunosuppressed mice, the immunosuppressant agents were discontinued, resulting in the complete rejection of iPSC-NS/PC-derived masses within 42 days after drug cessation. In accordance with the tumor rejection, hind-limb motor function was recovered in all of the mice. Moreover, infiltration of microglia and lymphocytes was observed during the course of tumor rejection, along with apoptosis of iPSC-NS/PC-generated cells. Thus, immune rejection can be used as a fail-safe system against potential tumorigenicity after transplantation of iPSC-NS/PCs to treat SCI.

Ivanov, V. N. and T. K. Hei "Regulation of viability, differentiation and death of human melanoma cells carrying neural stem cell biomarkers: a possibility for neural trans-differentiation." Apoptosis. 2015 Jul;20(7):996-1015. doi: 10.1007/s10495-015-1131-3.

During embryonic development, melanoblasts, the precursors of melanocytes, emerge from a subpopulation of the neural crest stem cells and migrate to colonize skin. Melanomas arise during melanoblast differentiation into melanocytes and from young proliferating melanocytes through somatic mutagenesis and epigenetic regulations. In the present study, we used several human melanoma cell lines from the sequential phases of melanoma development (radial growth phase, vertical growth phase and metastatic phase) to compare: (i) the frequency and efficiency of the induction of cell death via apoptosis and necroptosis; (ii) the presence of neural and cancer stem cell biomarkers as well as death receptors, DR5 and FAS, in both adherent and spheroid cultures of melanoma cells; (iii) anti-apoptotic effects of the endogenous production of cytokines and (iv) the ability of melanoma cells to perform neural trans-differentiation. We demonstrated that programed necrosis or necroptosis, could be induced in two metastatic melanoma lines, FEMX and OM431, while the mitochondrial pathway of apoptosis was prevalent in a vast majority of melanoma lines. All melanoma lines used in the current study expressed substantial levels of pluripotency markers, SOX2 and NANOG. There was a trend for increasing expression of Nestin, an early neuroprogenitor marker, during melanoma progression. Most of the melanoma lines, including WM35, FEMX and A375, can grow as a spheroid culture in serum-free media with supplements. It was possible to induce neural trans-differentiation of 1205Lu and OM431 melanoma cells in serum-free media supplemented with insulin. This was confirmed by the expression of neuronal markers, doublecortin and beta3-Tubulin, by significant growth of neurites and by the negative regulation of this process by a dominant-negative Rac1N17. These results suggest a relative plasticity of differentiated melanoma cells and a possibility for their neural trans-differentiation without the necessity for preliminary dedifferentiation.

Jankowiak, W., K. Kruszewski, et al. "Sustained Neural Stem Cell-Based Intraocular Delivery of CNTF Attenuates Photoreceptor Loss in the nclf Mouse Model of Neuronal Ceroid Lipofuscinosis." PLoS One. 2015 May 20;10(5):e0127204. doi: 10.1371/journal.pone.0127204. eCollection 2015.

A sustained intraocular administration of neurotrophic factors is among the strategies aimed at establishing treatments for currently untreatable degenerative retinal disorders. In the present study we have analyzed the neuroprotective effects of a continuous neural stem (NS) cell-based intraocular delivery of ciliary neurotrophic factor (CNTF) on photoreceptor cells in the nclf mouse, an animal model of the neurodegenerative lysosomal storage disorder variant late infantile neuronal ceroid lipofuscinosis (vLINCL). To this aim, we genetically modified adherently cultivated NS cells with a polycistronic lentiviral vector encoding a secretable variant of CNTF together with a Venus reporter gene (CNTF-NS cells). NS cells for control experiments (control-NS cells) were modified with a vector encoding the reporter gene tdTomato. Clonal CNTF-

NS and control-NS cell lines were established using fluorescent activated cell sorting and intravitreally grafted into 14 days old nclf mice at the onset of retinal degeneration. The grafted cells preferentially differentiated into astrocytes that were attached to the posterior side of the lenses and the vitreal side of the retinas and stably expressed the transgenes for at least six weeks, the latest post-transplantation time point analyzed. Integration of donor cells into host retinas, ongoing proliferation of grafted cells or adverse effects of the donor cells on the morphology of the host eyes were not observed. Quantitative analyses of host retinas two, four and six weeks after cell transplantation revealed the presence of significantly more photoreceptor cells in eyes with grafted CNTF-NS cells than in eyes with grafted control-NS cells. This is the first demonstration that a continuous intraocular administration of a neurotrophic factor attenuates retinal degeneration in an animal model of neuronal ceroid lipofuscinosis.

Ma, S. M., L. X. Chen, et al. "Periostin Promotes Neural Stem Cell Proliferation and Differentiation following Hypoxic-Ischemic Injury." PLoS One. 2015 Apr 20;10(4):e0123585. doi: 10.1371/journal.pone.0123585. eCollection 2015.

Neural stem cell (NSC) proliferation and differentiation are required to replace neurons damaged or lost after hypoxic-ischemic events and recover brain function. Periostin (POSTN), a novel matricellular protein, plays pivotal roles in the survival, migration, and regeneration of various cell types, but its function in NSCs of neonatal rodent brain is still unknown. The purpose of this study was to investigate the role of POSTN in NSCs following hypoxia-ischemia (HI). We found that POSTN mRNA levels significantly increased in differentiating NSCs. The proliferation and differentiation of NSCs in the hippocampus is compromised in POSTN knockout mice. Moreover, NSC proliferation and differentiation into neurons and astrocytes significantly increased in cultured NSCs treated with recombinant POSTN. Consistently, injection of POSTN into neonatal hypoxic-ischemic rat brains stimulated NSC proliferation and differentiation in the subventricular and subgranular zones after 7 and 14 days of brain injury. Lastly, POSTN treatment significantly improved the spatial learning deficits of rats subjected to HI. These results suggest that POSTN significantly enhances NSC proliferation and differentiation after HI, and provides new insights into therapeutic strategies for the treatment of hypoxic-ischemic encephalopathy.

Mandal, C., J. H. Park, et al. "Transcriptomic study of mouse embryonic neural stem cell differentiation under ethanol treatment." Mol Biol Rep. 2015 Jul;42(7):1233-9. doi: 10.1007/s11033-015-3862-1. Epub 2015 Feb 20.

Neural stem cells (NSCs) can be differentiated into one of three cell lineages: neurons, astrocytes or, oligodendrocytes. Some neurotoxins have the ability to deregulate this dynamic process. NSC cell fate can be altered by ethanol as reported previously. Our aim was to investigate the alteration of genes by ethanol during NSC differentiation and to explore the molecular mechanism underlying this phenomenon. Here, mouse fetal forebrain derived NSCs were differentiated for 2 days with or without of ethanol (50 mM). We performed a comparative microarray analysis at day two using GeneChip((R)) Mouse Genome 430A 2.0 arrays. Microarray analysis showed that the expressions of 496 genes were altered by ethanol (56 and 440 were up- and down-regulated, respectively). Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed the association of the following altered genes in the Wnt signaling pathway: Wnt5a, Csnk2a1, Tcf7l2, Ccnd2, Nlk, Tbl1x, Tbl1xr1, Rac2 and Nfatc3. Quantitative real time PCR analysis also demonstrated the relative expression levels of these genes. As Wnt signaling is a player of brain development, ethanol-induced alterations may contribute to improper development of the brain. Our data could be a useful resource for elucidating the mechanism behind the ethanol neurotoxicity in developing brain.

Marsh, J. C., S. Goldman, et al. "Involvement of the neural stem cell compartment by pediatric and adult gliomas: a retrospective review of 377 cases." J Neurooncol. 2015 Mar;122(1):105-10. doi: 10.1007/s11060-014-1682-0. Epub 2014 Dec 11.

To assess frequency of neural stem cell compartment (NSC) involvement in adult and pediatric gliomas [World Health Organization (WHO) grades 1-4], and to assess whether NSC involvement at presentation impacts on survival, recurrence rates, and/or transformation from low grade (WHO grade 1-2) to high grade disease (WHO grades 3-4). Cranial MRIs for 154 pediatric and 223 adult glioma patients treated from 2000 to 2012 were reviewed. NSC involvement was documented. Tumors were stratified by age (adult vs. pediatric), histology, tumor grade, tumor location, and involvement of midline structures. Odds ratios (OR) for death were calculated based on NSC status at presentation. Rates of transformation and recurrence rates (ORR) were compared using Fisher's Exact Test. Time to recurrence (TTR) was calculated using student t test. Among recurrent and transformed tumors, we also assessed the rate of NSC involvement at time of recurrence or transformation. 74.8 % of tumors had NSC involvement. Higher rates

of NSC involvement were seen among adult (p = .0001); high grade (p = .0001)); grade 2 versus grade 1 (p = .0001) and other grade 1 histologies (p = .0001) versus JPA (juvenile pilocytic astrocytoma) patients); grade 2-4 tumors (p = .0001); and supratentorial tumors (p < .0001). No transformation was noted among pediatric low grade tumors or adult grade 1 tumors. 22/119 (18.5 %) adult grade 2 tumors transformed. Rates of transformation were not impacted by NSC status (p = .47). ORR was 15.1 %, and was greater for NSC+ tumors at presentation (p = .05). 36/41 recurrences (87.8 %) involved NSC at time of recurrence. OR for death was 2.62 (1.16-5.9), p = .02 for NSC+ tumors at presentation. Adult and pediatric gliomas (all grades) frequently involve NSC at presentation, although rates are lower in pediatric JPA and all infratentorial tumors. NSC involvement at presentation increases OR death and reduces TTR for pediatric gliomas (all grades) and adult low grade gliomas, and shows a strong trend toward increased ORR.

Mateo, J. L., D. L. van den Berg, et al. "Characterization of the neural stem cell gene regulatory network identifies OLIG2 as a multifunctional regulator of self-renewal." Genome Res. 2015 Jan;25(1):41-56. doi: 10.1101/gr.173435.114. Epub 2014 Oct 7.

The gene regulatory network (GRN) that supports neural stem cell (NS cell) self-renewal has so far been poorly characterized. Knowledge of the central transcription factors (TFs), the noncoding gene regulatory regions that they bind to, and the genes whose expression they modulate will be crucial in unlocking the full therapeutic potential of these cells. Here, we use DNase-seq in combination with analysis of histone modifications to identify multiple classes of epigenetically and functionally distinct cis-regulatory elements (CREs). Through motif analysis and ChIP-seq, we identify several of the crucial TF regulators of NS cells. At the core of the network are TFs of the basic helix-loop-helix (bHLH), nuclear factor I (NFI), SOX, and FOX families, with CREs often densely bound by several of these different TFs. We use machine learning to highlight several crucial regulatory features of the network that underpin NS cell self-renewal and multipotency. We validate our predictions by functional analysis of the bHLH TF OLIG2. This TF makes an important contribution to NS cell self-renewal by concurrently activating pro-proliferation genes and preventing the untimely activation of genes promoting neuronal differentiation and stem cell quiescence.

Mazzini, L., M. Gelati, et al. "Human neural stem cell transplantation in ALS: initial results from a phase I trial." J Transl Med. 2015 Jan 27;13:17. doi: 10.1186/s12967-014-0371-2.

BACKGROUND: We report the initial results from a phase I clinical trial for ALS. We transplanted GMP-grade, fetal human neural stem cells from natural in utero death (hNSCs) into the anterior horns of the spinal cord to test for the safety of both cells and neurosurgical procedures in these patients. The trial was approved by the Istituto Superiore di Sanita and the competent Ethics Committees and was monitored by an external Safety Board. METHODS: Six non-ambulatory patients were treated. Three of them received 3 unilateral hNSCs microinjections into the lumbar cord tract, while the remaining ones received bilateral (n = 3 + 3) microinjections. None manifested severe adverse events related to the treatment, even though nearly 5 times more cells were injected in the patients receiving bilateral implants and a much milder immune-suppression regimen was used as compared to previous trials. RESULTS: No increase of disease progression due to the treatment was observed for up to18 months after surgery. Rather, two patients showed a transitory improvement of the subscore ambulation on the ALS-FRS-R scale (from 1 to 2). A third patient showed improvement of the MRC score for tibialis anterior, which persisted for as long as 7 months. The latter and two additional patients refused PEG and invasive ventilation and died 8 months after surgery due to the progression of respiratory failure. The autopsies confirmed that this was related to the evolution of the disease. CONCLUSIONS: We describe a safe cell therapy approach that will allow for the treatment of larger pools of patients for later-phase ALS clinical trials, while warranting good reproducibility. These can now be carried out under more standardized conditions, based on a more homogenous repertoire of clinical grade hNSCs. The use of brain tissue from natural miscarriages eliminates the ethical concerns that may arise from the use of fetal material. TRIAL REGISTRATION: EudraCT:2009-014484-39 .

Mooney, R., L. Roma, et al. "Neural stem cell-mediated intratumoral delivery of gold nanorods improves photothermal therapy." ACS Nano. 2014 Dec 23;8(12):12450-60. doi: 10.1021/nn505147w. Epub 2014 Nov 17.

Plasmonic photothermal therapy utilizes biologically inert gold nanorods (AuNRs) as tumor-localized antennas that convert light into heat capable of eliminating cancerous tissue. This approach has lower morbidity than surgical resection and can potentially synergize with other treatment modalities including chemotherapy and immunotherapy. Despite these advantages, it is still challenging to obtain

heating of the entire tumor mass while avoiding unnecessary collateral damage to surrounding healthy tissue. It is therefore critical to identify innovative methods to distribute an effective concentration of AuNRs throughout tumors without depositing them in surrounding healthy tissue. Here we demonstrate that AuNR-loaded, tumor-tropic neural stem cells (NSCs) can be used to improve the intratumoral distribution of AuNRs. A simple UV-vis technique for measuring AuNR loading within NSCs was established. It was then confirmed that NSC viability is unimpaired following AuNR loading and that NSCs retain AuNRs long enough to migrate throughout tumors. We then demonstrate that intratumoral injections of AuNR-loaded NSCs are more efficacious than free AuNR injections, as evidenced by reduced recurrence rates of triple-negative breast cancer (MDA-MB-231) xenografts following NIR exposure. Finally, we demonstrate that the distribution of AuNRs throughout the tumors is improved when transported by NSCs, likely resulting in the improved efficacy of AuNR-loaded NSCs as compared to free AuNRs. These findings highlight the advantage of combining cellular therapies and nanotechnology to generate more effective cancer treatments.

Nam, H., K. H. Lee, et al. "Adult human neural stem cell therapeutics: Current developmental status and prospect." World J Stem Cells. 2015 Jan 26;7(1):126-36. doi: 10.4252/wjsc.v7.i1.126.

Over the past two decades, regenerative therapies using stem cell technologies have been developed for various neurological diseases. Although stem cell therapy is an attractive option to reverse neural tissue damage and to recover neurological deficits, it is still under development so as not to show significant treatment effects in clinical settings. In this review, we discuss the scientific and clinical basics of adult neural stem cells (aNSCs), and their current developmental status as cell therapeutics for neurological disease. Compared with other types of stem cells, aNSCs have clinical advantages, such as limited proliferation, inborn differentiation potential into functional neural cells, and no ethical issues. In spite of the merits of aNSCs, difficulties in the isolation from the normal brain, and in the in vitro expansion, have blocked preclinical and clinical study using aNSCs. However, several groups have recently developed novel techniques to isolate and expand aNSCs from normal adult brains, and showed successful applications of aNSCs to neurological diseases. With new technologies for aNSCs and their clinical strengths, previous hurdles in stem cell therapies for neurological diseases could be overcome, to realize clinically efficacious regenerative stem cell therapeutics.

Nudi, E. T., J. Jacqmain, et al. "Combining Enriched Environment, Progesterone, and Embryonic Neural Stem Cell Therapy Improves Recovery after Brain Injury." J Neurotrauma. 2015 Mar 18.

Millions of persons every year are affected by traumatic brain injury (TBI), and currently no therapies have shown efficacy in improving outcomes clinically. Recent research has suggested that enriched environments (EE), embryonic neural stem cells (eNSC), and progesterone (PROG) improve functional outcomes after TBI, and further, several investigators have suggested that a polytherapuetic approach may have greater efficacy than a single therapy. The purpose of the current study was to determine if varying combinations of post-injury EE, progesterone therapy, or eNSC transplantation would improve functional outcomes over just a single therapy. A controlled cortical impact was performed in rats to create a lesion in the medial frontal cortex. The rats were then placed in either EE or standard environments and administered 10 mg/kg progesterone or vehicle injections 4 h post-injury and every 12 h for 72 h after the initial injection. Seven days after the surgery, rats were transplanted with either eNSCs or media. Rats were then tested on the open field test, Barnes maze, Morris water maze, and Rotor-Rod tasks. Improved functional outcomes were shown on a majority of the behavioral tasks in animals that received a combination of therapies. This effect was especially prominent with therapies that were combined with EE. Immunohistochemistry showed that the transplanted eNSCs survived, migrated, and displayed neural phenotypes. These data suggest that a poly-therapeutic approach after TBI improves functional recovery to a greater magnitude. Moreover, when polytherapies are combined with EE, the effects on recovery are enhanced, leading to greater recovery of function.

Perrigue, P. M., M. E. Silva, et al. "The histone demethylase jumonji coordinates cellular senescence including secretion of neural stem cell-attracting cytokines." Mol Cancer Res. 2015 Apr;13(4):636-50. doi: 10.1158/1541-7786.MCR-13-0268. Epub 2015 Feb 4.

Jumonji domain-containing protein 3 (JMJD3/KDM6B) demethylates lysine 27 on histone H3 (H3K27me3), a repressive epigenetic mark controlling chromatin organization and cellular senescence. To better understand the functional consequences of JMJD3 its expression was investigated in brain tumor cells. Querying patient expression profile databases confirmed JMJD3 overexpression in high-grade glioma. Immunochemical staining of two glioma cell lines,

U251 and U87, indicated intrinsic differences in JMJD3 expression levels that were reflected in changes in cell phenotype and variations associated with cellular senescence, including senescence-associated beta-galactosidase (SA-beta-gal) activity and the senescence-associated secretory phenotype (SASP). Overexpressing wild-type JMJD3 (JMJD3wt) activated SASP-associated genes, enhanced SA-beta-gal activity, and induced nuclear blebbing. Conversely, overexpression of a catalytically inactive dominant negative mutant JMJD3 (JMJD3mut) increased proliferation. In addition, a large number of transcripts were identified by RNA-seq as altered in JMJD3 overexpressing cells, including cancer- and inflammation-related transcripts as defined by Ingenuity Pathway Analysis. These results suggest that expression of the SASP in the context of cancer undermines normal tissue homeostasis and contributes to tumorigenesis and tumor progression. These studies are therapeutically relevant because inflammatory cytokines have been linked to homing of neural stem cells and other stem cells to tumor loci. IMPLICATIONS: This glioma study brings together actions of a normal epigenetic mechanism (JMJD3 activity) with dysfunctional activation of senescence-related processes, including secretion of SASP proinflammatory cytokines and stem cell tropism toward tumors.

Pires, F., Q. Ferreira, et al. "Neural stem cell differentiation by electrical stimulation using a cross-linked PEDOT substrate: Expanding the use of biocompatible conjugated conductive polymers for neural tissue engineering." Biochim Biophys Acta. 2015 Jun;1850(6):1158-68. doi: 10.1016/j.bbagen.2015.01.020. Epub 2015 Feb 7.

BACKGROUND: The use of conjugated polymers allows versatile interactions between cells and flexible processable materials, while providing a platform for electrical stimulation, which is particularly relevant when targeting differentiation of neural stem cells and further application for therapy or drug screening. METHODS: Materials were tested for cytotoxicity following the ISO10993-5. PEDOT: PSS was cross-linked. ReNcellVM neural stem cells (NSC) were seeded in laminin coated surfaces, cultured for 4days in the presence of EGF (20ng/mL), FGF-2 (20ng/mL) and B27 (20mug/mL) and differentiated over eight additional days in the absence of those factors under 100Hz pulsed DC electrical stimulation, 1V with 10ms pulses. NSC and neuron elongation aspect ratio as well as neurite length were assessed using ImageJ. Cells were immune-stained for Tuj1 and GFAP. RESULTS: F8T2, MEH-PPV, P3HT and cross-linked PEDOT: PSS (x PEDOT: PSS) were assessed as non-cytotoxic. L929 fibroblast population was 1.3 higher for x PEDOT: PSS than for glass control, while F8T2 presents moderate proliferation. The population of neurons (Tuj1) was 1.6 times higher with longer neurites (73 vs 108mum) for cells cultured under electrical stimulus, with cultured NSC. Such stimulus led also to longer neurons. CONCLUSIONS: x PEDOT: PSS was, for the first time, used to elongate human NSC through the application of pulsed current, impacting on their differentiation towards neurons and contributing to longer neurites. GENERAL SIGNIFICANCE: The range of conductive conjugated polymers known as non-cytotoxic was expanded. x PEDOT: PSS was introduced as a stable material, easily processed from solution, to interface with biological systems, in particular NSC, without the need of in-situ polymerization.

Ravindran, G. and H. Devaraj "Prognostic significance of neural stem cell markers, Nestin and Musashi-1, in oral squamous cell carcinoma: expression pattern of Nestin in the precancerous stages of oral squamous epithelium." Clin Oral Investig. 2015 Jul;19(6):1251-60. doi: 10.1007/s00784-014-1341-z. Epub 2014 Oct 29.

BACKGROUND: Besides the tissue-specific stem cell markers, neural and hematopoietic stem cell markers were found to play an important role in carcinogenesis. Based on this background, we have investigated the expression pattern and prognostic significance of neural stem cell markers, Nestin and Musashi-1, in oral cancer. METHODS: We used immunohistochemistry and immunofluorescence analyses to study the expression pattern and correlation between Nestin and Musashi-1 in oral squamous cell carcinoma. The Kaplan-Meier method was used to construct overall and disease-free survival curves, and the differences were calculated using log-rank test. RESULTS: Nestin expression was gradually increased in the transformation stages of oral cancer. Both Nestin and Musashi-1 expressions were associated with higher stage and poorly differentiated status of oral carcinoma. Interestingly, Nestin and Musashi-1 double positive cases showed statistically highly significant correlation with poorer survival of oral carcinoma patients. CONCLUSIONS: Expression of Nestin in the preneoplastic lesions indicates its role in the transformation of oral squamous epithelium. Clinicopathological and survival analyses suggest that Nestin and Musashi-1 might be associated with invasion, differentiation and poorer survival in oral squamous cell carcinoma. In addition to their role as independent prognostic indicators, Nestin and Musashi-1 double positivity can be used to select high-risk cases for effective therapy and this is the novel finding of this study. CLINICAL

RELEVANCE: Nestin and Musashi-1 are found to be independent prognostic markers of oral cancer, and they might be used as molecular targets for effective therapy.

Shirasaka, T. and S. Kurosawa "[Potential therapy of intravenous neural stem cell transplantation for psychiatric disorder--a strategy for facilitation of neural network and behavioral recovery]." Nihon Arukoru Yakubutsu Igakkai Zasshi. 2014 Oct;49(5):259-69.

Recent clinical neuroimaging studies have revealed a possible relationship between morphological brain changes and the manifestation of psychiatric disorders such as depression, schizophrenia, and alcoholism. Although its biological mechanism is still unclear, the emerging evidence suggests that the alteration of neurogenesis is the key factor for the morphological brain changes of these psychiatric disorders. In our previous work, we analyzed the mechanism of neural network disruption by ethanol using cultured cells, and found a suppressive effect of ethanol on neural stem cell (NSC) differentiation. While, we also demonstrated that antidepressants, mood stabilizers and atypical antipsychotics stimulate NSC differentiation which was inhibited by ethanol. In the present work, we have demonstrated that the usefulness of intravenous transplantation of NSCs to fetal alcohol spectrum disorder (FASD) model rat for the purpose of reconstructing the impaired neural network and investigating the possibility of regenerative therapy for patients with neurobehavioral deficits of FASD. We have shown the potential migration of transplanted NSCs into the brain by visualizing a fluorescent cell marker and radioisotope, as well as the possible recovery of behavioral abnormalities observed in FASD model rats, such as memory/cognitive function, and social interaction. We further assessed the characteristics of transplanted cells in the brain and found that the GABAergic interneurons were increased in amygdale, DG, cingulated cortex areas in the model rat. In the amygdala and cingulate Cortex of model rats, number of parvalbumin positive cells was reduced and the NSC transplantation recovered these disturbances. Moreover, in the amygdala and cingulate cortex, intravenous NSC transplantation appears to regenerate expression of post-synaptic density protein 95 (PSD95) in FASD model rats. These results indicate that intravenous NSC transplantation has the potential to become a therapeutic intervention for FASD patients.

Shirazi, H. A., J. Rasouli, et al. "1,25-Dihydroxyvitamin D3 enhances neural stem cell proliferation and oligodendrocyte differentiation." Exp Mol Pathol. 2015 Apr;98(2):240-5. doi: 10.1016/j.yexmp.2015.02.004. Epub 2015 Feb 11.

1,25-Dihydroxyvitamin D3 (1,25(OH)2D3) has recently been found to suppress experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Although its effect was attributed to an anti-inflammatory mechanism, it is not clear whether this treatment can also directly act on neural cells to promote CNS recovery. The present study investigates the effect of various concentrations of 1,25(OH)2D3 on neural stem cell (NSC) proliferation and their differentiation to oligodendrocytes, the myelinating cells. We have, for the first time, shown that NSCs constitutively express vitamin D receptor (VDR), which can be upregulated by 1,25(OH)2D3. This vitamin significantly enhanced proliferation of NSCs, and enhanced their differentiation into neurons and oligodendrocytes, but not astrocytes. NSCs treated with 1,25(OH)2D3 showed increased expression of NT-3, BDNF, GDNF and CNTF, important neurotrophic factors for neural cell survival and differentiation. Overall, we demonstrated that 1,25(OH)2D3 has a direct effect on NSC proliferation, survival, and neuron/oligodendrocyte differentiation, thus representing a novel mechanism underlying its remyelinating and neuroprotective effect in MS/EAE therapy.

Sims, B., L. Gu, et al. "Neural stem cell-derived exosomes mediate viral entry." Int J Nanomedicine. 2014 Oct 21;9:4893-7. doi: 10.2147/IJN.S70999. eCollection 2014.

BACKGROUND: Viruses enter host cells through interactions of viral ligands with cellular receptors. Viruses can also enter cells in a receptor-independent fashion. Mechanisms regarding the receptor-independent viral entry into cells have not been fully elucidated. Exosomal trafficking between cells may offer a mechanism by which viruses can enter cells. METHODS: To investigate the role of exosomes on cellular viral entry, we employed neural stem cell-derived exosomes and adenovirus type 5 (Ad5) for the proof-of-principle study. RESULTS: Exosomes significantly enhanced Ad5 entry in Coxsackie virus and adenovirus receptor (CAR)-deficient cells, in which Ad5 only had very limited entry. The exosomes were shown to contain T-cell immunoglobulin mucin protein 4 (TIM-4), which binds phosphatidylserine. Treatment with anti-TIM-4 antibody significantly blocked the exosome-mediated Ad5 entry. CONCLUSION: Neural stem cell-derived exosomes mediated significant cellular entry of Ad5 in a receptor-independent fashion. This mediation may be hampered by an antibody specifically targeting

TIM-4 on exosomes. This set of results will benefit further elucidation of virus/exosome pathways, which would contribute to reducing natural viral infection by developing therapeutic agents or vaccines.

Smith, I., V. Silveirinha, et al. "Human neural stem cell-derived cultures in three-dimensional substrates form spontaneously functional neuronal networks." J Tissue Eng Regen Med. 2015 Feb 25. doi: 10.1002/term.2001.

Differentiated human neural stem cells were cultured in an inert three-dimensional (3D) scaffold and, unlike two-dimensional (2D) but otherwise comparable monolayer cultures, formed spontaneously active, functional neuronal networks that responded reproducibly and predictably to conventional pharmacological treatments to reveal functional, glutamatergic synapses. Immunocytochemical and electron microscopy analysis revealed a neuronal and glial population, where markers of neuronal maturity were observed in the former. Oligonucleotide microarray analysis revealed substantial differences in gene expression conferred by culturing in a 3D vs a 2D environment. Notable and numerous differences were seen in genes coding for neuronal function, the extracellular matrix and cytoskeleton. In addition to producing functional networks, differentiated human neural stem cells grown in inert scaffolds offer several significant advantages over conventional 2D monolayers. These advantages include cost savings and improved physiological relevance, which make them better suited for use in the pharmacological and toxicological assays required for development of stem cell-based treatments and the reduction of animal use in medical research. Copyright (c) 2015 John Wiley & Sons, Ltd.

Stevanato, L., C. Hicks, et al. "Differentiation of a Human Neural Stem Cell Line on Three Dimensional Cultures, Analysis of MicroRNA and Putative Target Genes." J Vis Exp. 2015 Apr 12;(98). doi: 10.3791/52410.

Neural stem cells (NSCs) are capable of self-renewal and differentiation into neurons, astrocytes and oligodendrocytes under specific local microenvironments. In here, we present a set of methods used for three dimensional (3D) differentiation and miRNA analysis of a clonal human neural stem cell (hNSC) line, currently in clinical trials for stroke disability (NCT01151124 and NCT02117635, Clinicaltrials.gov). HNSCs were derived from an ethical approved first trimester human fetal cortex and conditionally immortalized using retroviral integration of a single copy of the c-mycER(TAM)construct. We describe how to measure axon process outgrowth of hNSCs differentiated on 3D scaffolds and how to quantify associated changes in miRNA expression using PCR array. Furthermore we exemplify computational analysis with the aim of selecting miRNA putative targets. SOX5 and NR4A3 were identified as suitable miRNA putative target of selected significantly down-regulated miRNAs in differentiated hNSC. MiRNA target validation was performed on SOX5 and NR4A3 3'UTRs by dual reporter plasmid transfection and dual luciferase assay.

Steward, O., K. G. Sharp, et al. "Characterization of ectopic colonies that form in widespread areas of the nervous system with neural stem cell transplants into the site of a severe spinal cord injury." J Neurosci. 2014 Oct 15;34(42):14013-21. doi: 10.1523/JNEUROSCI.3066-14.2014.

We reported previously the formation of ectopic colonies in widespread areas of the nervous system after transplantation of fetal neural stem cells (NSCs) into spinal cord transection sites. Here, we characterize the incidence, distribution, and cellular composition of the colonies. NSCs harvested from E14 spinal cords from rats that express GFP were treated with a growth factor cocktail and grafted into the site of a complete spinal cord transection. Two months after transplant, spinal cord and brain tissue were analyzed histologically. Ectopic colonies were found at long distances from the transplant in the central canal of the spinal cord, the surface of the brainstem and spinal cord, and in the fourth ventricle. Colonies were present in 50% of the rats, and most rats had multiple colonies. Axons extended from the colonies into the host CNS. Colonies were strongly positive for nestin, a marker for neural precursors, and contained NeuN-positive cells with processes resembling dendrites, GFAP-positive astrocytes, APC/CC1-positive oligodendrocytes, and Ki-67-positive cells, indicating ongoing proliferation. Stereological analyses revealed an estimated 21,818 cells in a colony in the fourth ventricle, of which 1005 (5%) were Ki-67 positive. Immunostaining for synaptic markers (synaptophysin and VGluT-1) revealed large numbers of synaptophysin-positive puncta within the colonies but fewer VGluT-1 puncta. Continuing expansion of NSC-derived cell masses in confined spaces in the spinal cord and brain could produce symptoms attributable to compression of nearby tissue. It remains to be determined whether other cell types with self-renewing potential can also form colonies.

Tennstaedt, A., M. Aswendt, et al. "Human neural stem cell intracerebral grafts show spontaneous early neuronal differentiation after several weeks."

Biomaterials. 2015 Mar;44:143-54. doi: 10.1016/j.biomaterials.2014.12.038. Epub 2015 Jan 12.

Human neural stem cells (hNSCs) hold great promise for the treatment of neurological diseases. Considerable progress has been made to induce neural differentiation in the cell culture in vitro and upon transplantation in vivo [2] in order to explore restoration of damaged neuronal circuits. However, in vivo conventional strategies are limited to post mortem analysis. Here, we apply our developed first fate mapping platform to monitor neuronal differentiation in vivo by magnetic resonance imaging, bioluminescence imaging, and fluorescence imaging. Ferritin, Luciferase and GFP under neuronal-specific promoters for immature and mature neurons, respectively, were used to generate transgenic hNSCs. Differentiation-linked imaging reporter expression was validated in vitro. The time profile of spontaneous neuronal maturation after transplantation into mouse brain cortex demonstrated early neuronal differentiation within 6 weeks. Fully mature neurons expressing synaptogenesis were observed only after three months or longer. Our trimodal fate mapping strategy represents a unique non-invasive tool to monitor the time course of neuronal differentiation of transplanted stem cells in vivo.

Theocharidis, U., K. Long, et al. "Regulation of the neural stem cell compartment by extracellular matrix constituents." Prog Brain Res. 2014;214:3-28. doi: 10.1016/B978-0-444-63486-3.00001-3.

Neural stem cells (NSCs) derive from the neuroepithelium of the neural tube, develop into radial glial cells, and recede at later developmental stages. In the adult, late descendants of these embryonic NSCs reside in discretely confined areas of the central nervous system, the stem cell niches. The best accepted canonical niches are the subventricular zone of the lateral ventricle and the subgranular zone of the dentate gyrus of the hippocampus. Stem cell niches provide a privileged environment to NSCs that supports self-renewal and maintenance of this cellular compartment. While numerous studies have highlighted the importance of transcription factors, morphogens, cytokines, and growth factors as intrinsic and extrinsic factors of stem cell regulation, less attention has been paid to the molecular micromilieu that characterizes the stem cell niches. In this chapter, we summarize increasing evidence that the extracellular matrix (ECM) of the stem cell environment is of crucial importance for the biology of this cellular compartment. A deeper understanding of the molecular composition of the ECM, the complementary receptors, and the signal transduction pathways engaged may prove highly relevant for harnessing NSCs in the context of biotechnological applications.

Wang, D. and J. Zhang "Effects of hypothermia combined with neural stem cell transplantation on recovery of neurological function in rats with spinal cord injury." Mol Med Rep. 2015 Mar;11(3):1759-67. doi: 10.3892/mmr.2014.2905. Epub 2014 Nov 10.

The microenvironment of the injured spinal cord is hypothesized to be involved in driving the differentiation and survival of engrafted neural stem cells (NSCs). Hypothermia is known to improve the microenvironment of the injured spinal cord in a number of ways. To investigate the effect of NSC transplantation in combination with hypothermia on the recovery of rat spinal cord injury, 60 Sprague Dawley female rats were used to establish a spinal cord hemisection model. They were divided randomly into three groups: A, spinal cord injury group; B, NSC transplantation group; and C, NSC transplantation + hypothermia group. At 1, 2, 4, 6 and 8 weeks postinjury, the motor function of all animals was evaluated using the Basso, Beattie and Besnaham locomotor scoring system and the inclined plane test. At 4 weeks posttransplantation, histological analysis and immunocytochemistry were performed. At 8 weeks posttransplantation, horseradish peroxidase nerve tracing and transmission electron microscopy were conducted to observe axonal regeneration. The outcome of hind limb motor function recovery in group C significantly surpassed that in group B at 4 weeks postinjury ($P<0.05$). Recovery was also observed in group A, but to a lesser degree. For the pathological sections no neural axonal were observed in group A. A few axonlike structures were observed in group B and more in group C. Horseradish peroxidaselabeled neurofibers and bromodeoxyuridinepositive cells were observed in the spinal cords of group C. Fewer of these cells were found in group B and fewer still in group A. The differences among the three groups were significant ($P<0.05$). Using transmission electron microscopy, newly formed nerve fibers and myelinated nerve fibers were observed in the central transverse plane in groups B and C, although these nerve fibers were not evident in group A. In conclusion, NSC transplantation promoted the recovery of hind limb function in rats, and combination treatment with hypothermia produced synergistic effects.

Wei, L., J. Wang, et al. "Hyperbaric oxygenation promotes neural stem cell proliferation and protects the learning and memory ability in neonatal hypoxic-ischemic brain damage." Int J Clin Exp Pathol. 2015 Feb 1;8(2):1752-9. eCollection 2015.

The aim of our study was to evaluate whether hyperbaric oxygenation (HBO) was an effective therapy for neonatal hypoxic ischemic brain damage (HIBD). Seven-day-old rat pups were divided into 3 groups: sham, hypoxia-ischemia (HI) control and HI-HBO group. HBO was administered for HI rats daily. The pathologic changes in brain tissues were observed by hematoxylin-eosin (H-E) staining. The immunohistochemical staining was applied to detect the Nestin and 5-bromo-2-deoxyuridine (BrdU) positive cells in hippocampal dentate gyrus region. The learning and memory function of rats was examined by Morris water maze. The HI rats showed obvious pathologic changes accompanied by levels decreasing and disorder arrangement of pyramidal cells, glial cells proliferation in postoperative, and nerve nuclei broken, while pathologic changes of rats in sham group was approximate to that in the HI + HBO group that was opposite to the HI group. Compared with the sham group, the Nestin and BrdU positive cells in HBO + HI group at different time points increased significantly ($P < 0.01$). Learning and memory function of rats in HI group was poor compared with the sham/HI + HBO group ($P < 0.01$), while that in HI + HBO group was approximate to that in sham group ($P > 0.05$). HBO treatment improved the learning and memory ability of the HI rats. HBO therapy may be effective for neonatal HIBD treatment.

Wu, Q., H. Zhang, et al. "[Neural stem cell-specific peroxisome proliferator-activated receptor gamma knockout mice: breeding and genetic identification]." Nan Fang Yi Ke Da Xue Xue Bao. 2014 Dec;34(12):1768-71.

OBJECTIVE: To breed neual stem cell-specific peroxisome proliferator-activated receptor gamma (PPARgamma) knockout mice. METHODS: Two transgenic mouse models, namely B6.PPARgammaloxp/loxp and B6.Nestin-Cre were interbred, and the first- generation offsprings were backcrossed with B6.PPARgammaloxp/loxp to obtain the second-generation mice. Genomic DNA was extracted from the second-generation mice for PCR to amplify the loxp and Cre gene fragments followed by agarose gel electrophoresis to verify their sizes. The mice with the PPARgammaloxp/loxp.Nestin-Cre (KO) genotype were selected as the neural stem cell-specific knockout PPARgamma mice, with B6.PPARgammaloxp/loxp (loxp) mice as the control. Tissue samples were collected from specific regions of the mouse brain and peripheral tissue for detecting the expression of PPARgamma mRNA using RT-PCR and real-time quantitative PCR. RESULTS AND CONCLUSION: Genotyping results showed PPARgammaloxp and Cre bands in the knockout mice, which showed obviously decreased mRNA expression of PPARgamma, suggesting successful establishment of neural stem cell-specific PPARgamma knockout mice. The two transgenic mice we used were fertile, and their breeding pattern followed the laws of Mendelian inheritance.

Yan, Y. M., X. L. Wang, et al. "Metabolites from the mushroom Ganoderma lingzhi as stimulators of neural stem cell proliferation." Phytochemistry. 2015 Apr 13. pii: S0031-9422(15)00125-9. doi: 10.1016/j.phytochem.2015.03.013.

Ganoderma lingzhi is a valuable, edible and medicinal fungus that has been widely used for the prevention and treatment of a broad range of diseases. In this study, spirolingzhines A-D, four meroterpenoids with a spiro[benzofuran-2,1'-cyclopentane] motif, lingzhines A-F, six meroterpenoids with diverse ring systems, along with two known compounds were isolated from the fruiting bodies of this fungus. The structures and stereochemistry of these substances were determined by using spectroscopic, X-ray crystallographic and computational methods. Chiral HPLC was used to separate (-)- and (+)-antipodes of seven of ten meroterpenoids, which were isolated from the fungus as racemic mixtures. Several of the metabolites were found to promote proliferation of neural stem cells (NSCs) and, as such, they constitute a class of NSC stimulators. The most potent member of this series, (-)-spirolingzhine A, was shown to affect NSC cell cycle progression using the 5-bromo-2-deoxyuridine (BrdU) incorporation assay.

Zhang, M., Y. Chai, et al. "Synergistic effects of Buyang Huanwu decoction and embryonic neural stem cell transplantation on the recovery of neurological function in a rat model of spinal cord injury." Exp Ther Med. 2015 Apr;9(4):1141-1148. Epub 2015 Feb 2.

The aim of the present study was to investigate the therapeutic effect of a combined treatment of Buyang Huanwu decoction (BYHWD), a well-known formula of traditional Chinese medicine, and neural stem cells (NSCs) on spinal cord injury (SCI) and the associated underlying mechanisms. A SCI model was established by surgery via a complete transection of the T10 vertebra of female Sprague-Dawley rats. Gelatin sponges were used to absorb NSCs labeled with the thymidine analog, 5-bromo-2-deoxyuridine (BrdU), and were transferred into the transected spinal cords. BYHWD was administered once a day by introgastric infusion. Motor functions of the hind limbs were evaluated using the 21-point locomotor rating scale developed by Basso, Beattie and Bresnahan (BBB). The fate of the transplanted

NSCs under the various conditions was examined by double immunofluorescence staining, using markers for neurons, astrocytes and oligodendrocytes, with BrdU. Ultrastructural changes of the SCI site following the various treatments were examined under a transmission electron microscope. The number of double positive cells for glial fibrillary acidic protein and BrdU in the BYHWD + NSC group was significantly decreased when compared with that in the NSC group ($P<0.05$). However, the number of cells that were labeled double positive for myelin basic protein and BrdU, as well as neuron specific enolase and BrdU, was greater in the BYHWD + NSC group when compared with the NSC group. Electron microscopy demonstrated that treatment with BYHWD combined with NSCs significantly alleviated demyelination. Results from the BBB motor function test exhibited a significant improvement in the BYHWD + NSC group when compared with the SCI, BYHWD and NSC only groups. In conclusion, the results demonstrated that the traditional Chinese medicine formula, BYHWD, exerted an effect on the differentiation and migration of NSCs. Combining the administration of BYHWD with NSCs was shown to have a synergistic effect on the recovery of neurological function, mitigating the progress of demyelination or ameliorating the recovery of myelination.

Zhang, W., G. J. Gu, et al. "Neural stem cell transplantation enhances mitochondrial biogenesis in a transgenic mouse model of Alzheimer's disease-like pathology." Neurobiol Aging. 2015 Mar;36(3):1282-92. doi: 10.1016/j.neurobiolaging.2014.10.040. Epub 2014 Dec 18.

Mitochondrial dysfunction, especially a defect in mitochondrial biogenesis, is an early and prominent feature of Alzheimer's disease (AD). Previous studies demonstrated that the number of mitochondria is significantly reduced in susceptible hippocampal neurons from AD patients. Neural stem cell (NSC) transplantation in AD-like mice can compensate for the neuronal loss resulting from amyloid-beta protein deposition. The effects of NSC transplantation on mitochondrial biogenesis and cognitive function in AD-like mice, however, are poorly understood. In this study, we injected NSCs or vehicle into 12-month-old amyloid precursor protein (APP)/PS1 transgenic mice, a mouse model of AD-like pathology. The effects of NSC transplantation on cognitive function, the amount of mitochondrial DNA, the expression of mitochondrial biogenesis factors and mitochondria-related proteins, and mitochondrial morphology were investigated. Our results show that in NSC-injected APP/PS1 (Tg-NSC) mice, the cognitive function, number of mitochondria, and expression of mitochondria-related proteins, specifically the mitochondrial fission factors (dynamin-related protein 1 [Drp1] and fission 1 [Fis1]) and the mitochondrial fusion factor optic atrophy 1 (OPA1), were significantly increased compared with those in age-matched vehicle-injected APP/PS1 (Tg-Veh) mice, whereas the expression of mitochondrial fusion factors mitofusion 1 (Mfn1) and Mfn2 was significantly decreased. These data indicate that NSC transplantation may enhance mitochondria biogenesis and further rescue cognitive deficits in AD-like mice.

Zizkova, M., R. Sucha, et al. "Proteome-wide analysis of neural stem cell differentiation to facilitate transition to cell replacement therapies." Expert Rev Proteomics. 2015 Feb;12(1):83-95. doi: 10.1586/14789450.2015.977381. Epub 2014 Nov 3.

Neurodegenerative diseases are devastating disorders and the demands on their treatment are set to rise in connection with higher disease incidence. Knowledge of the spatiotemporal profile of cellular protein expression during neural differentiation and definition of a set of markers highly specific for targeted neural populations is a key challenge. Intracellular proteins may be utilized as a readout for follow-up transplantation and cell surface proteins may facilitate isolation of the cell subpopulations, while secreted proteins could help unravel intercellular communication and immunomodulation. This review summarizes the potential of proteomics in revealing molecular mechanisms underlying neural differentiation of stem cells and presents novel candidate proteins of neural subpopulations, where understanding of their functionality may accelerate transition to cell replacement therapies.

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