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Engineering Therapeutic Neural Stem Cell Lines for Parkinson's Disease

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1. Introduction

The isolation of tissue-specific self-renewable stem cells from human embryonic or adult stem cells is one of the most promising applications for regenerative medicine. Ongoing research suggests that multipotent stem cells are a viable source of specialized cells for tissue repair. Undifferentiated stem cells are better suited when multiple cell types are lost to injury or disease. However, when a single lineage species is associated with an injury or disease, multipotent stem cells can be instructed to terminally differentiate into specific cell types. The terminal differentiation is induced by culturing the multipotent stem cells in media containing specific instructive molecules or by over-expressing lineage-specific gene(s). Further studies are needed to generate cellular phenotypes with stable expression in vitro and after grafting into diseased or injured tissue. Harnessing mechanisms governing tissue histogenesis that take place during early embryogenesis is a promising strategy for engineering specific cell types or tissues. Likewise, the same developmental pathways could also be induced in vitro using a set of instructive cues different from those normally involved during embryonic development.
Within the framework of developing therapeutic products, this chapter will discuss the cellular and molecular control of neural stem cell derivation from adult and pluripotent stem cells and their differentiation into dopaminergic lineage.

2. Derivation and properties of neural stem cells

Neural stem cells (NSCs) are defined by their ability to self-renew and generate a large number of progeny able to differentiate into the principal central nervous system (CNS) cell types: neurons, astrocytes and oligodendrocytes. NSCs have the ability to maintain themselves in culture under genetic or epigenetic stimulation and to generate a large number of progeny. Contrary to the hematopoietic system where the hematopoietic stem cells are defined by a set of cell markers and thus can be purified by fluorescence-activated cell sorting (FACS), there are no specific cellular markers, necessary and sufficient to identify NSCs. The cell surface marker CD133 (prominin-1/2) epitope has been used to isolate the neurosphere-forming neural precursors from human fetal brain (Uchida et al.,

2000). However, this cell surface marker is also expressed by immature hematopoietic stem cells, epithelial, tumor cells and endothelial progenitors characterized by the expression of other cell surface markers including CD34 and CD45 (Miraglia et al., 1998; Corbeil et al., 2000; Peichev et al., 2000; Florek et al., 2005). Thus, to purify the neural precursor population it has to be sorted out from the CD34 and CD45 negative population. Using a combinatorial surface antigen code, specific populations of neural precursors are isolated from adult, fetal brain or pluripotent human embryonic stem cells. A recent study used the same combination of cell surface markers to isolate neural stem cells from hESCs (Golebiewska et al., 2009). This population of neural precursors expressed low levels of the pluripotency markers Oct4 and Nanog (Johansson et al., 1999) CD133+ cells expressed the neural specific marker Pax6, nestin, vimentin, Olig2, Sox1, sox3, Gli3, beta-tubulin3 and myelin basic protein (MBP). Interestingly these cells also expressed the transcription factor Pax7 that is characteristic of muscle precursor cells (Golebiewska et al., 2009). This mixed expression pattern suggests that cells do not correlate with a specific regional identity and that CD133+ cells perhaps retain the potential to differentiate into a wide range of cells.

A second cell surface marker expressed by neural precursors in vivo in the subventricular zone (SCZ) is CD15 (stage-specific embryonic antigen-1, lewis –X antigen) (Capela and Temple, 2002). CD15+ SVZ-derived precursors give rise to neurospheres capable of differentiating into all major CNS cell types. In this study, the CD15- / CD24+ ependymal cells lining the 3rd ventricle, previously thought to contribute to the NSC compartment (Johansson et al., 1999), were incapable of forming neurospheres in vitro (Capela and Temple, 2002).

In an effort to isolate homogenous populations of neural stem cells from hESCs, a combination of markers was used simultaneously. This combinatorial flow cytometry included the cell surface markers CD15+, CD29 high (small cell lung carcinoma cluster 4 antigen) and CD24 low (beta1-integrin) (Pruszak et al., 2009). CD15 is strongly expressed in Sox1+ and Sox2+ neurepithelial rosette structures derived from hESCs, as well in the CD133+ cells. The CD15+/$CD24_{LO}$/$CD29_{HI}$ subset was enriched for the neurosphere forming colonies. Interestingly, transplantation of this cell type showed neuroepithelial tumors that displayed characteristic neural rosettes expressing neural precursor markers: Sox2, nestin, vimentin and radial glial markers 3CB2 and RC2. The expression profile of the CD15–/$CD24_{LO}$/$CD29_{HI}$ fraction was characteristic of the neural crest/mesenchymal stem cells and expressed the cell surface markers CD271, CD57 and CD73. This subpopulation was also tumorigenic after injection in animals. The third subpopulation with the CD15–/$CD24_{HI}$/$CD29_{LO}$ surface antigen signature defined a neuronal/neuroblast population that was highly enriched for neuronal markers, such as doublecortin and microtubule-associated protein (MAP)-2. In contrast, CD15–/$CD24_{HI}$/$CD29_{LO}$ grafts did not form tumors, differentiated into NCAM-positive cells and extended neuronal processes into the host brain (Pruszak et al., 2009).

Forse1 (forebrain surface embryonic antigen-1) is another cell surface marker expressed by neural precursors (Tole et al., 1995) and used to identify multipotent neural stem cells (Tole et al., 1995; Pruszak et al., 2007; Elkabetz et al., 2008). Cells expressing Forse1 within the hESC-derived neural rosettes exhibited anterior neural fate as assessed by the expression of the forebrain transcription factor BF1. Forse1- cells gave rise to neural crest stem cells and were enriched for posterior CNS markers. Interestingly, both Force1+ and Force1- retain the ability to form rosettes and Force1+ have the potential to revert to caudal fates, including

spinal cord motor neurons and midbrain dopamine neurons and to generate neural crest cells (Elkabetz et al., 2008). Transplantation of the Force1+ neural precursors into the rat striatum led to graft overgrowth and formation of rosette in vivo. This overgrowth was observed even when Force1+ cells were sorted before transplantation suggesting that cell contamination is not the cause of the tumorigenicity.

A recent study, however, demonstrated that neither CD133 nor CD15 are necessary markers to define a neural stem cell. Human NSCs positive or negative for either CD133 or CD15 have exhibited multipotency and the ability to differentiate into neurons, astrocytes and oligodendrocytes (Sun et al., 2009). Of interest, the CD133 marker appears to be down regulated as the cells enter the S phase and during the G2 or M phases. This observation renders the neural stem cell identity even more elusive.

In addition to cell surface markers, specific populations of NSCs may be isolated based on their responsiveness to mitogenic growth factors. The fundamental three properties necessary for cells to be defined as NSC are: 1) self-renewal ability and maintenance of long-term cultures through multiple passages under clonogenic conditions, 2) generation of a large number of progenies and 3) differentiation into the three principal neural lineages i.e. neurons, astrocytes and oligodendrocytes.

Conti et al. isolated homogenous and clonogenic populations of NSCs from mouse and human ESC-derived rosettes (Conti et al., 2005). The rosettes were mechanically transferred into serum free media in the presence of EGF and bFGF and propagated for up to 5 months. When exposed to appropriate differentiation factors, these NSCs expressed nestin, vimentin and the radial glial marker 3CB2 and differentiated into neurons, astrocytes and oligodendrocytes, (Sun et al., 2008). After transplantation into adult mouse hippocampus and striatum, these NSCs engrafted and differentiated into neurons and astrocytes without forming tumors.

Daadi et al. recently reported the isolation and perpetuation of a homogenous population of hNSCs, from hECSs based on their proliferative response to the exposure to EGF, bFGF and LIF (Daadi et al., 2008). The cumulative cell number and population doubling analysis demonstrated the continuous and stable growth of the hNSCs. These hNSCs were clonogenic and expressed the neural precursor cell markers nestin, vimentin and the radial glial cell marker 3CB2. Under differentiation conditions, the hNSCs gave rise to neurons, astrocytes and oligodendrocytes, expressed transcripts for the neural-specific genes nestin, Notch1 and neural cell adhesion molecule (N-CAM), Sox2 and for the lineage specific markers β-tubulin class III, medium-size neurofilament (NF-M) and microtubule-associated protein 2 (MAP-2) for neurons, GFAP for astrocytes and myelin basic protein (MBP) for oligodendrocytes.

Koch et al. recently reported the isolation, perpetuation and characterization of the rosette-derived EGF+FGF2 responsive hNSCs (Koch et al., 2009). These neural precursors were isolated from rosettes dissected out of the culture plate and grown in suspension as spheres. In this study the self-renewable NSCs were maintained for up to 75 passages without apparent changes in proportions of the neural lineages and a pronounced differentiation toward neuronal lineage (40 to 70%). These NSCs developed an anterior hindbrain identity with predominant generation of GABAergic neurons. They retained the ability to convert to a ventral midbrain identity in response to sonic hedgehog (SHH) and FGF8 treatment with 31% of beta-tubulin+ neurons expressing tyrosine hydroxylase.

Another approach used to generate a homogeneous and specific NSC population at the clonal level is the genetic immortalization of neural precursors with propagating genes, such as v-myc, large T-antigen and telomerase reverse transcriptase (hTER) (Snyder et al., 1992; Whittemore and Synder, 1996; Lundberg et al., 1997; Roy et al., 2004). Over expression of human telomerase reverse transcriptase (hTERT) was used to immortalize neural progenitors from the human fetal spinal cord. These cells have been shown to yield multiple cell lines with different lineage proportions including some restricted to a neuronal lineage both *in vitro* and *in vivo*. The cell line expressed markers consistent with a ventral spinal neuronal (interneurons and motor neurons) phenotype. The functional property of the neurons was demonstrated electrophysiologically by using calcium influx in response to depolarizing stimuli. The cells were passaged without evidence of senescence, karyotypic abnormality or loss of normal growth control. The cells did not form tumors or overgrow after transplantation into developing rat fetal telencephalon or spinal cord. Human NSC clones were genetically propagated using v-myc (Flax et al., 1998; Villa et al., 2009). Transplantation of these hNSCs demonstrated their ability to migrate throughout the CNS and differentiate into multiple developmentally and regionally appropriate cell types. The gene product of v-myc was undetectable in grafted hNSCs 24-48 hours following transplantation, which suggests the lack of graft overgrowth in vivo. However, there is possibility of clonal variations in v-myc expression or re-activation of v-myc in vivo and tumor formation. Thus, regulated expression of immortalizing genes would be a safer approach for exploring therapeutic application of the immortalized cell lines (Hoshimaru et al., 1996).

The prospective isolation and perpetuation of homogenous populations of neural stem cells have also been carried out using reporter genes placed under the regulatory control of cell-specific promoters. Using human cells, this technique requires the transfection and FACS isolation of the cell population expressing the reporter gene, such as green fluorescent protein (GFP). Among the cell-specific gene promoters used to isolate neural stem cells are nestin, musashi, Sox1 and Sox2. Nestin is an intermediate filament expressed by neuroepithelial stem cells. The second intronic enhancer of nestin directs its transcription to neural stem and progenitor cells. This strategy was used to isolate homogenous nestin+ neural stem cells and to differentiate them into specific lineages (Keyoung et al., 2001). Musashi1 is an RNA-binding protein expressed by neural progenitors of the fetal brain (Kaneko et al., 2000). Neural cells expressing the musashi1/hGFP co-expressed nestin in 96% of the progeny. The majority of cells (93%) are undergoing cell division as monitored by BrdU incorporation (Keyoung et al., 2001). Sox1 gene is one of the earliest genes that mark the neuroectoderm specification in the developing mouse embryo. It is expressed in neuroepithelial precursors but down-regulated during neuronal and glial differentiation. Using a Sox1-GFP knock-in line, purified populations of neural stem cells were isolated, perpetuated and differentiated into specialized neuronal populations. (Ying et al., 2003; Barraud et al., 2005; Chung et al., 2006). Similarly, Zappone et al defined the regulatory element of Sox2 gene expression in both stem and progenitor cells (Zappone et al., 2000). Using adenoviral vector expressing Sox2/EGFP Wang et al transduced and FACS purified Sox2 expressing neural stem cells from the developing human fetal brain (Wang et al.). The Sox2+ neural precursors were self-renewable, multipotent and displayed higher telomerase enzymatic activity, in comparison to the Sox2-depleted population.

3. Therapeutic application in Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopamine (DA) neurons in the substantia nigra pars compacta, resulting in decreased dopaminergic input to the striatum. Symptoms include tremors, rigidity, bradykinesia and instability. Existing therapies for PD are only palliative and treat the symptoms but do not address the underlying cause or prevent the progression of the disease. Levodopa (L-dopa), the gold standard pharmacological treatment to restore dopamine, is compromised over time by decreased efficacy and by increased side effects. Neurosurgical treatments, such as pallidotomy, thalamotomy and deep electrical stimulation are only considered after the failure of pharmacological treatment. A reliable long-term treatment to halt the progression of the disease and restore function remains elusive.

Neural transplantation is a promising strategy for improving dopaminergic dysfunction in PD. Over 20 years of research using fetal mesencephalic tissue as a source of DA neurons has demonstrated the therapeutic potential of cell transplantation therapy in rodents and non-human primate animal models and in human patients (Mendez et al., 2008). In many patients grafts have survived, formed synaptic connections and improved motor function (Olanow et al., 1997; Barker and Dunnett, 1999). However, there are limitations associated with human fetal tissue transplantation, including high tissue variability, lack of scalability, ethical concerns and inability to obtain an epidemiologically meaningful quantity of tissue. Thus, the control of the identity, purity and potency of these cells becomes exceedingly difficult and jeopardizes both the safety of the patient and the efficacy of the therapy. With a reliance on fetal tissue as a source of neurons, cell replacement therapy cannot develop into a widely available treatment option for patients with neurodegenerative diseases. These critical issues render the search and development of alternative sources of cells a very worthwhile goal with societal importance and commercial application.

4. Differentiation of neural stem cells into dopaminergic neurons

Alternative sources of natural dopamine expressing cells explored have been the adrenal medulla cells (Schueler et al., 1993), PC12 cells (Ono et al., 1997), the glomus cells of the carotid bodies (Espejo et al., 1998) and the porcine fetal tissue (Deacon et al., 1997). Most of these sources have been abandoned due to poor cell survival, inefficiency or health risks for the patient (Yurek and Sladek, 1990; Isacson and Breakefield, 1997). The current most promising strategy in generating an unlimited supply of cells for neural transplantation is the generation of dopaminergic neurons from NSCs.

Cellular differentiation may be defined as a multistep process driving a given cell from a precursor stage to functional competence. These steps often are manifested by changes in cellular morphology and by the appearance of new gene products. Each differentiation step is timely orchestrated and often depends on the interplay between the cell's intrinsic and extrinsic programs. Knowledge of both extrinsic differentiation signals and the molecular machinery underlying the intrinsic events is rapidly progressing.

Extrinsic cues may regulate neuronal diversity by selectively rescuing a specific subpopulation of neuronal precursors committed to expressing a specific neurotransmitter phenotype or by instructing the neuronal precursors during a narrow developmental window to adopt a specific fate.

The relative distribution of the *in vivo* environmental cues is thought to play a critical role in directing fate choices of stem cell neuronal progeny. For instance, in the peripheral nervous

system (PNS), neural crest stem cells (NCSCs) derived from the E10.5 neural tube behave differently from the E14.5 sciatic nerve-derived NCSC. The latter became less sensitive to the autonomic instructive action of bone morphogenetic protein-2 (BMP-2) and consequently their potential is limited to a cholinergic fate. This time-dependant decrease in the BMP-2 sensitivity may have resulted from a combination of an *in vivo* selection and developmental change in the NCSCs mode to respond to BMP2.

A promising stem cell source for DA neurons is embryonic stem (ES) cells. Early studies demonstrated that these cells have the potential to generate DA neurons (Kawasaki et al., 2000; Lee et al., 2000a). In presence of serum, ES cells form clusters of floating cells or embryoid bodies (EBs) containing ectodermal, mesodermal and endodermal derivatives. When these EBs are treated with FGF2, FGF8 and Shh, 71% of the cells differentiated into neurons as identified with the neuronal marker class III β-tubulin and 33% of these neurons displayed characteristics of the midbrain DA neurons (Lee et al., 2000a). A second group of investigators proceeded first to generate a homogenous neural lineage from the ES cells (Kawasaki et al., 2000) before inducing DA phenotype. This was achieved by co-culturing ES cells with the stromal cells PA6 that induced the pan-neural marker NCAM in 92% of the ES cells colonies. PA6-derived conditioned media was inefficient in inducing neural differentiation, but was not blocked by a 0.4 μm membrane barrier. Paradoxically, paraformaldehyde fixed PA6 cells retained the inductive activity. Under these culture conditions, 52% of differentiated cells expressed neuronal markers and 30% of these neurons assumed midbrain DA phenotype. These DA induced neurons appear to engraft after implantation and to improve behavioral deficits of 6-OHDA lesioned mice. Mesencephalic explant cultures studies (Baizabal and Covarrubias, 2009) showed that ES-derived neural precursors exhibit a limited developmental window to respond to the midbrain DA cues and that FGF8 +SHH treatment promotes commitment to DA lineage.

There has been a concerted effort to isolate a stable, expandable stem cell from the midbrain, based on the hypothesis that the progeny will be destined or at least inducible to become the A9 class of the midbrain projecting DA neurons and differentiate exclusively into nigro-striatal-like DA neurons. Early studies have demonstrated that EGF responsive precursor cells do exist within the midbrain, however, these progeny did not consistently or robustly, differentiate into DA neurons neither *in vitro* (Mytilineou et al., 1992; Svendsen et al., 1995; Potter et al., 1999) nor after implantation into the rat striatum (Svendsen et al., 1996; Svendsen et al., 1997). Interestingly, IL-1 induced TH expression in the midbrain-derived progenitors (Potter et al., 1999). In addition, membrane-bound factors potentiated the TH induction and stimulated morphological maturation in these progenitors (Ptak et al., 1995; Ling et al., 1998). The continuous generation of DA neurons from a long-term expandable midbrain-derived stem cell will require the development of processes for proliferation and maintenance of DA-specific precursors. Noteworthy, radial glia of the floor plate can give rise to the midbrain DA neurons *in vivo* (Bonilla et al., 2008). In addition, ascorbic acid and lowered oxygen concentration appear to support survival and proliferation of DA neurons, respectively (Studer et al., 2000; Yan et al., 2001). The effect of a low oxygen level (3±2%) was partially mimicked by erythropoietin (Epo). A different approach (Sawamoto et al., 2001) consisted of FACS sorting mesencephalic precursors according to their expression of GFP driven by nestin enhancer. Nestin is a neurofilament, expressed by neuroepithelial stem cells (Lendahl et al., 1990). The nestin-GFP+ precursors were clonally analyzed and shown to have the ability to self renew and generate clusters of progeny able to differentiate into neurons, astrocytes and oligodendrocytes. Among this neuronal population TH+ neurons

were identified with no particular treatment. Importantly, five weeks after implantation of the sorted GFP+ cells into the striatum of 6-OHDA hemiparkinsonian rats, the animals showed reduction in amphetamine induced rotation. Using this FACS approach, midbrain DA radial glial-like precursors were isolated at the embryonic age E10.5, based on the expression of Lmx1a and the floor plate marker Corin (Jonsson et al., 2009). This study and others highlight the therapeutic efficacy of the A9 DA progenitors in cell transplantation therapy for PD. As follow up to the 2 early studies described above (Kawasaki et al., 2000; Lee et al., 2000b) numerous reports have described techniques of generating dopaminergic neurons from hESCs (Schulz et al., 2004; Zeng et al., 2004; Park et al., 2005; Yan et al., 2005; Sonntag et al., 2007; Cho et al., 2008). Some approaches require co-culturing with stromal cells, human astrocytes, meningeal, sertoli cells or others (Kawasaki et al., 2000; Buytaert-Hoefen et al., 2004; Perrier et al., 2004; Takagi et al., 2005; Roy et al., 2006; Yue et al., 2006; Chiba et al., 2008; Hayashi et al., 2008). Studies have now begun to decipher the active components responsible for the DA phenotype induction. For instance, a recent study demonstrated that the DA-inductive signals of the stromal cell line was mimicked by the combination of a defined set of factors, including stromal cell-derived factor 1, pleiotrophin, insulin-like growth factor 2 and ephrin B1 (Vazin et al., 2009). The inducing factors in the other cell lines and signaling pathways involved in the DA specification remain to be determined.

5. Conclusions

Neural stem cells offer us a great tool for understanding the basic biology of cell fate choices and allow us to explore novel inducing factors and new developmental networks of gene cascades that may not necessarily occur under *in vivo* physiological conditions. A deeper and broader understanding of the molecular and cellular functioning in the development of specialized neural cells, strengthens our ability to efficiently produce stable, pure and viable sources of DA neurons. Ideally this knowledge will also enlighten the next step when cellular products are tested *in vivo* and pre-clinical efficacy is determined. Among challenging issues in product development for PD are the cell line stability, scalability, composition, efficiency in DA neurons generation, viability, cryo-preservation, recovery, identity, purity, potency and the *in vivo* fate of the implanted cell. Thus, given the complexity of neural system, long-term translational research will play an important and critical role in developing safe and efficacious cellular products for treating PD patients.

6. References

Baizabal JM, Covarrubias L (2009) The embryonic midbrain directs neuronal specification of embryonic stem cells at early stages of differentiation. Dev Biol 325:49-59.

Barker RA, Dunnett SB (1999) Functional integration of neural grafts in Parkinson's disease. Nat Neurosci 2:1047-1048.

Barraud P, Thompson L, Kirik D, Bjorklund A, Parmar M (2005) Isolation and characterization of neural precursor cells from the Sox1-GFP reporter mouse. Eur J Neurosci 22:1555-1569.

Bonilla S, Hall AC, Pinto L, Attardo A, Gotz M, Huttner WB, Arenas E (2008) Identification of midbrain floor plate radial glia-like cells as dopaminergic progenitors. Glia 56:809-820.

Buytaert-Hoefen KA, Alvarez E, Freed CR (2004) Generation of tyrosine hydroxylase positive neurons from human embryonic stem cells after coculture with cellular substrates and exposure to GDNF. Stem Cells 22:669-674.

Capela A, Temple S (2002) LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. Neuron 35:865-875.

Chiba S, Lee YM, Zhou W, Freed CR (2008) Noggin enhances dopamine neuron production from human embryonic stem cells and improves behavioral outcome after transplantation into Parkinsonian rats. Stem Cells 26:2810-2820.

Cho MS, Lee YE, Kim JY, Chung S, Cho YH, Kim DS, Kang SM, Lee H, Kim MH, Kim JH, Leem JW, Oh SK, Choi YM, Hwang DY, Chang JW, Kim DW (2008) Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci U S A 105:3392-3397.

Chung S, Shin BS, Hedlund E, Pruszak J, Ferree A, Kang UJ, Isacson O, Kim KS (2006) Genetic selection of sox1GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. J Neurochem 97:1467-1480.

Conti L, Pollard SM, Gorba T, Reitano E, Toselli M, Biella G, Sun Y, Sanzone S, Ying QL, Cattaneo E, Smith A (2005) Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. PLoS Biol 3:e283.

Corbeil D, Roper K, Hellwig A, Tavian M, Miraglia S, Watt SM, Simmons PJ, Peault B, Buck DW, Huttner WB (2000) The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. J Biol Chem 275:5512-5520.

Daadi MM, Maag AL, Steinberg GK (2008) Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLoS ONE 3:e1644.

Deacon T, Schumacher J, Dinsmore J, Thomas C, Palmer P, Kott S, Edge A, Penney D, Kassissieh S, Dempsey P, Isacson O (1997) Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nat Med 3:350-353.

Elkabetz Y, Panagiotakos G, Al Shamy G, Socci ND, Tabar V, Studer L (2008) Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. Genes Dev 22:152-165.

Espejo EF, Montoro RJ, Armengol JA, Lopez-Barneo J (1998) Cellular and functional recovery of Parkinsonian rats after intrastriatal transplantation of carotid body cell aggregates. Neuron 20:197-206.

Flax JD, Aurora S, Yang C, Simonin C, Wills AM, Billinghurst LL, Jendoubi M, Sidman RL, Wolfe JH, Kim SU, Snyder EY (1998) Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. Nature Biotechnology 16:1033-1039.

Florek M, Haase M, Marzesco AM, Freund D, Ehninger G, Huttner WB, Corbeil D (2005) Prominin-1/CD133, a neural and hematopoietic stem cell marker, is expressed in adult human differentiated cells and certain types of kidney cancer. Cell Tissue Res 319:15-26.

Golebiewska A, Atkinson SP, Lako M, Armstrong L (2009) Epigenetic landscaping during hESC differentiation to neural cells. Stem Cells 27:1298-1308.

Hayashi H, Morizane A, Koyanagi M, Ono Y, Sasai Y, Hashimoto N, Takahashi J (2008) Meningeal cells induce dopaminergic neurons from embryonic stem cells. Eur J Neurosci 27:261-268.

Hoshimaru M, Ray J, Sah DWY, Gage FH (1996) Differentiation of the immortalized adult neuronal progenitor cell line HC2S2 into neurons by regulatable suppression of the v-myc oncogene. Proceedings of the National Academy of Sciences USA 93:1518-1523.

Isacson O, Breakefield XO (1997) Benefits and risks of hosting animal cells in the human brain [see comments]. Nat Med 3:964-969.

Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J (1999) Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96:25-34.

Jonsson ME, Ono Y, Bjorklund A, Thompson LH (2009) Identification of transplantable dopamine neuron precursors at different stages of midbrain neurogenesis. Exp Neurol 219:341-354.

Kaneko Y, Sakakibara S, Imai T, Suzuki A, Nakamura Y, Sawamoto K, Ogawa Y, Toyama Y, Miyata T, Okano H (2000) Musashi1: an evolutionally conserved marker for CNS progenitor cells including neural stem cells. Dev Neurosci 22:139-153.

Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y (2000) Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron 28:31-40.

Keyoung HM, Roy NS, Benraiss A, Louissaint A, Jr., Suzuki A, Hashimoto M, Rashbaum WK, Okano H, Goldman SA (2001) High-yield selection and extraction of two promoter-defined phenotypes of neural stem cells from the fetal human brain. Nat Biotechnol 19:843-850.

Koch P, Opitz T, Steinbeck JA, Ladewig J, Brustle O (2009) A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc Natl Acad Sci U S A 106:3225-3230.

Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD (2000a) Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. Nat Biotechnol 18:675-679.

Lee SH, Lumelsky N, Studer L, Auerbach JM, McKay RD (2000b) Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. Nat Biotechnol 18:675-679.

Lendahl U, Zimmerman LB, McKay RD (1990) CNS stem cells express a new class of intermediate filament protein. Cell 60:585-595.

Ling ZD, Potter ED, Lipton JW, Carvey PM (1998) Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines. Exp Neurol 149:411-423.

Lundberg C, Martinez-Serrano A, Cattaneo E, McKay RD, Bjorklund A (1997) Survival, integration, and differentiation of neural stem cell lines after transplantation to the adult rat striatum. Experimental Neurology 145:342-360.

Mendez I, Vinuela A, Astradsson A, Mukhida K, Hallett P, Robertson H, Tierney T, Holness R, Dagher A, Trojanowski JQ, Isacson O (2008) Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years. Nat Med 14:507-509.

Miraglia S, Godfrey W, Buck D (1998) A response to AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? Blood 91:4390-4391.

Mytilineou C, Park TH, Shen J (1992) Epidermal growth factor-induced survival and proliferation of neuronal precursor cells from embryonic rat mesencephalon. Neurosci Lett 135:62-66.

Olanow CW, Freeman TB, Kordower JH (1997) Neural transplantation as a therapy for Parkinson's disease. Adv Neurol 74:249-269.

Ono T, Date I, Imaoka T, Shingo T, Furuta T, Asari S, Ohmoto T (1997) Evaluation of intracerebral grafting of dopamine-secreting PC12 cells into allogeneic and xenogeneic brain. Cell Transplant 6:511-513.

Park CH, Minn YK, Lee JY, Choi DH, Chang MY, Shim JW, Ko JY, Koh HC, Kang MJ, Kang JS, Rhie DJ, Lee YS, Son H, Moon SY, Kim KS, Lee SH (2005) In vitro and in vivo analyses of human embryonic stem cell-derived dopamine neurons. J Neurochem 92:1265-1276.

Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 95:952-958.

Perrier AL, Tabar V, Barberi T, Rubio ME, Bruses J, Topf N, Harrison NL, Studer L (2004) Derivation of midbrain dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci U S A 101:12543-12548.

Potter ED, Ling ZD, Carvey PM (1999) Cytokine-induced conversion of mesencephalic-derived progenitor cells into dopamine neurons. Cell Tissue Res 296:235-246.

Pruszak J, Sonntag KC, Aung MH, Sanchez-Pernaute R, Isacson O (2007) Markers and Methods for Cell Sorting of Human Embryonic Stem Cell-Derived Neural Cell Populations. Stem Cells 25:2257-2268.

Pruszak J, Ludwig W, Blak A, Alavian K, Isacson O (2009) CD15, CD24, and CD29 define a surface biomarker code for neural lineage differentiation of stem cells. Stem Cells 27:2928-2940.

Ptak LR, Hart KR, Lin D, Carvey PM (1995) Isolation and manipulation of rostral mesencephalic tegmental progenitor cells from rat. Cell Transplant 4:335-342.

Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA (2006) Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat Med 12:1259-1268.

Roy NS, Nakano T, Keyoung HM, Windrem M, Rashbaum WK, Alonso ML, Kang J, Peng W, Carpenter MK, Lin J, Nedergaard M, Goldman SA (2004) Telomerase immortalization of neuronally restricted progenitor cells derived from the human fetal spinal cord. Nat Biotechnol 22:297-305.

Sawamoto K, Nakao N, Kakishita K, Ogawa Y, Toyama Y, Yamamoto A, Yamaguchi M, Mori K, Goldman SA, Itakura T, Okano H (2001) Generation of dopaminergic neurons in the adult brain from mesencephalic precursor cells labeled with a nestin-GFP transgene. J Neurosci 21:3895-3903.

Schueler SB, Ortega JD, Sagen J, Kordower JH (1993) Robust survival of isolated bovine adrenal chromaffin cells following intrastriatal transplantation: a novel hypothesis of adrenal graft viability. J Neurosci 13:4496-4510.

Schulz TC, Noggle SA, Palmarini GM, Weiler DA, Lyons IG, Pensa KA, Meedeniya AC, Davidson BP, Lambert NA, Condie BG (2004) Differentiation of human embryonic stem cells to dopaminergic neurons in serum-free suspension culture. Stem Cells 22:1218-1238.

Snyder EY, Deitcher DL, Walsh C, Arnold-Aldea S, Hartwieg EA, Cepko CL (1992) Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. Cell 68:33-51.

Sonntag KC, Pruszak J, Yoshizaki T, van Arensbergen J, Sanchez-Pernaute R, Isacson O (2007) Enhanced yield of neuroepithelial precursors and midbrain-like dopaminergic neurons from human embryonic stem cells using the bone morphogenic protein antagonist noggin. Stem Cells 25:411-418.

Studer L, Csete M, Lee SH, Kabbani N, Walikonis J, Wold B, McKay R (2000) Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. J Neurosci 20:7377-7383.

Sun Y, Kong W, Falk A, Hu J, Zhou L, Pollard S, Smith A (2009) CD133 (Prominin) negative human neural stem cells are clonogenic and tripotent. PLoS One 4:e5498.

Sun Y, Pollard S, Conti L, Toselli M, Biella G, Parkin G, Willatt L, Falk A, Cattaneo E, Smith A (2008) Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture. Mol Cell Neurosci 38:245-258.

Svendsen CN, Fawcett JW, Bentlage C, Dunnett SB (1995) Increased survival of rat EGF-generated CNS precursor cells using B27 supplemented medium. Exp Brain Res 102:407-414.

Svendsen CN, Clarke DJ, Rosser AE, Dunnett SB (1996) Survival and differentiation of rat and human epidermal growth factor- responsive precursor cells following grafting into the lesioned adult central nervous system. Exp Neurol 137:376-388.

Svendsen CN, Caldwell MA, Shen J, ter Borg MG, Rosser AE, Tyers P, Karmiol S, Dunnett SB (1997) Long-term survival of human central nervous system progenitor cells transplanted into a rat model of Parkinson's disease. Exp Neurol 148:135-146.

Takagi Y, Takahashi J, Saiki H, Morizane A, Hayashi T, Kishi Y, Fukuda H, Okamoto Y, Koyanagi M, Ideguchi M, Hayashi H, Imazato T, Kawasaki H, Suemori H, Omachi S, Iida H, Itoh N, Nakatsuji N, Sasai Y, Hashimoto N (2005) Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model. J Clin Invest 115:102-109.

Tole S, Kaprielian Z, Ou SK, Patterson PH (1995) FORSE-1: a positionally regulated epitope in the developing rat central nervous system. J Neurosci 15:957-969.

Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, Tsukamoto AS, Gage FH, Weissman IL (2000) Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci U S A 97:14720-14725.

Vazin T, Becker KG, Chen J, Spivak CE, Lupica CR, Zhang Y, Worden L, Freed WJ (2009) A novel combination of factors, termed SPIE, which promotes dopaminergic neuron differentiation from human embryonic stem cells. PLoS One 4:e6606.

Villa A, Liste I, Courtois ET, Seiz EG, Ramos M, Meyer M, Juliusson B, Kusk P, Martinez-Serrano A (2009) Generation and properties of a new human ventral mesencephalic neural stem cell line. Exp Cell Res 315:1860-1874.

Wang S, Chandler-Militello D, Lu G, Roy NS, Zielke A, Auvergne R, Stanwood N, Geschwind D, Coppola G, Nicolis SK, Sim FJ, Goldman SA Prospective

identification, isolation, and profiling of a telomerase-expressing subpopulation of human neural stem cells, using sox2 enhancer-directed fluorescence-activated cell sorting. J Neurosci 30:14635-14648.

Whittemore SR, Synder EY (1996) Physiological relevance and functional potential of central nervous system-derived cell lines. Molecular Neurobiology 12:13-38.

Yan J, Studer L, McKay RD (2001) Ascorbic acid increases the yield of dopaminergic neurons derived from basic fibroblast growth factor expanded mesencephalic precursors. J Neurochem 76:307-311.

Yan Y, Yang D, Zarnowska ED, Du Z, Werbel B, Valliere C, Pearce RA, Thomson JA, Zhang SC (2005) Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. Stem Cells 23:781-790.

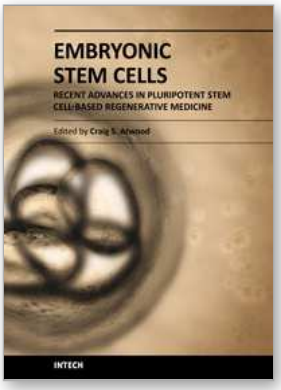
Ying QL, Stavridis M, Griffiths D, Li M, Smith A (2003) Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nat Biotechnol 21:183-186.

Yue F, Cui L, Johkura K, Ogiwara N, Sasaki K (2006) Induction of midbrain dopaminergic neurons from primate embryonic stem cells by coculture with sertoli cells. Stem Cells 24:1695-1706.

Yurek DM, Sladek JR, Jr. (1990) Dopamine cell replacement: Parkinson's disease. Annu Rev Neurosci 13:415-440.

Zappone MV, Galli R, Catena R, Meani N, De Biasi S, Mattei E, Tiveron C, Vescovi AL, Lovell-Badge R, Ottolenghi S, Nicolis SK (2000) Sox2 regulatory sequences direct expression of a (beta)-geo transgene to telencephalic neural stem cells and precursors of the mouse embryo, revealing regionalization of gene expression in CNS stem cells. Development 127:2367-2382.

Zeng X, Cai J, Chen J, Luo Y, You ZB, Fotter E, Wang Y, Harvey B, Miura T, Backman C, Chen GJ, Rao MS, Freed WJ (2004) Dopaminergic differentiation of human embryonic stem cells. Stem Cells 22:925-940.



Embryonic Stem Cells - Recent Advances in Pluripotent Stem Cell-Based Regenerative Medicine

Edited by Prof. Craig Atwood

ISBN 978-953-307-198-5
Hard cover, 410 pages
Publisher InTech
Published online 26, April, 2011
Published in print edition April, 2011

Pluripotent stem cells have the potential to revolutionise medicine, providing treatment options for a wide range of diseases and conditions that currently lack therapies or cures. This book describes recent advances in the generation of tissue specific cell types for regenerative applications, as well as the obstacles that need to be overcome in order to recognize the potential of these cells.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Marcel M. Daadi (2011). Engineering Therapeutic Neural Stem Cell Lines for Parkinson's Disease, Embryonic Stem Cells - Recent Advances in Pluripotent Stem Cell-Based Regenerative Medicine, Prof. Craig Atwood (Ed.), ISBN: 978-953-307-198-5, InTech, Available from: http://www.intechopen.com/books/embryonic-stem-cells-recent-advances-in-pluripotent-stem-cell-based-regenerative-medicine/engineering-therapeutic-neural-stem-cell-lines-for-parkinson-s-disease

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