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13

# Neural Stem/Progenitor Cell Clones as Models for Neural Development and Transplantation

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## 1. Introduction

Neural stem cells (NSCs) are somatic stem cells, capable of giving rise to all the mature cell types of the adult nervous system. NSCs are generally "simple" in their cell shape, and acquire much more complex morphology as they differentiate into mature cell types. Morphological changes are not the only phenomena, both genetic and epigenetic alterations also mark the NSC differentiation, allowing development of series of genetic and immunological markers to identify and isolate certain cell types from developing neural tissue. During early phase of the development of central nervous system (CNS), NSCs occupy the newly formed neural tube as a thin layer of cells (called neuroepithelium), which later become thicker and more complex in cellular architecture by the process of cell proliferation, differentiation, migration and maturation in a precisely organized fashion. The underlying mechanisms of these processes are always the attraction to neurobiologists.

NSC differentiation does not occur overnight; rather, it occurs in a progressive manner hypothesized by the lineage-restriction theory. As development proceeds, NSCs acquire additional features (demonstrated by markers) and become regionally diversified under the influence of gradients of growth factors and cytokines. These cells still proliferate, but their differentiation potential may be limited capable of giving rise to fewer cell types as they divide. This group of dividing cells at any CNS developmental stages is often referred as "neural progenitor pool" (Fig. 1). Lineage restriction hypothesis indicates that NSCs undergo progressive loss of differentiation potential (i.e. the number of cell types that they can give rise to) as neural development proceeds. In support of this notion, certain cell surface markers (e.g. A2B5 and 5A5) have been found to label and fractionate lineage-restricted progenitors of the developing spinal cord in a non-overlapping manner (Mayer-Proschel et al., 1997). However, lineage restriction is not the only mechanism of NSC development. For example, Noble examined the expression patterns of A2B5 and 5A5 in the developing forebrain and identified a population of neural progenitors expressing both markers suggesting that NSC differentiation is a much more complicated process in the

brain than in the spinal cord (Noble et al., 2003). Therefore, the original lineage-restriction theory based on the expression of A2B5 and 5A5 may be an oversimplified one and can not be generalized to explain NSC differentiation in the entire developing CNS. Nevertheless, it initiated a logical prediction, which will be modified or corrected by more upcoming results from different area of the developing CNS.

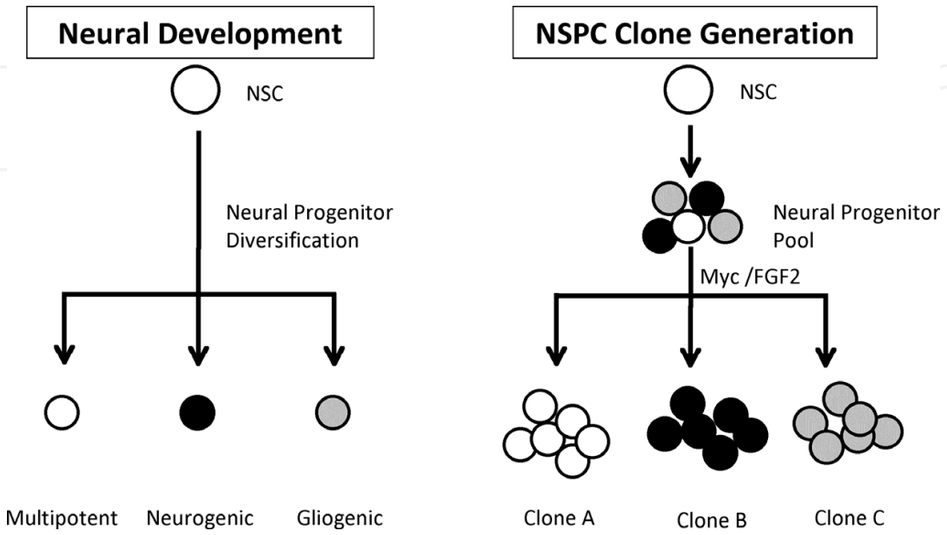


Fig. 1. Immortalized NSPC clones represent neural progenitor diversity during development.

NSCs differentiate at different rate and direction, which creates a diversity of neural progenitors at any given period of CNS development. One way to reveal neural progenitor diversity is to generate neural progenitor clones (Fig. 1). Over the past decades, numerous NSC and neural progenitor clones have been generated, and they have proven to be great models in delineating mechanisms of NSC differentiation. Many of these clones have also been transplanted into animal models of various neurological diseases and trauma, and demonstrated their great usefulness for obtaining mechanistic insights on how exogenous NSCs interact with host tissues and promising effects in animal's behavioural recovery. In our laboratory, we also took this approach and generated various neural stem/progenitor cell (NSPC) clones from rat embryonic forebrains. In this book chapter, we will review more recent discoveries with these "old" and more recently generated neural progenitor clones as in vitro models to unravel NSC differentiation mechanisms as well as cellular tools for testing their therapeutic potentials in paradigms of transplantation. In the era of translational research, we would like to stress on the need for neurobiologists to take on the mission seriously and format our thinking to materialize the transition from bench to bedside.

## 2. Generation of neural stem/progenitor cell clones

Neural cell lines have been generated by numerous approaches including isolation from spontaneous or induced neural tumors and somatic cell fusion with immortal cells. These cell lines have been widely used as in vitro models, some of which we are still experimenting on today such as Neuro-2a, PC12 cells. More recently, with the advance of our understanding on genetic networks controlling cell proliferation, the "purpose-driven"

generation of NSPC clones has been systematically performed. Both growth factor stimulation (epigenetic) and viral introduction of immortalizing genes such as oncogenes (genetic) have proven to be efficient in generating NSPC clones. In this section, we will summarize genetic functions of commonly used immortalizing genes (Myc, neu, large T-antigen, adenoviral E1A, Tert and p53) in promoting cell proliferation and growth factor dependency of the resulting NSPC clones including human NSPC clones (summarized in Table 1 below).

### 2.1 Immortalizing genes

Myc has been most extensively studied in cancer research since its deregulation relates many different types of tumors (Grandori et al., 2000). Myc also plays a critical role in stem cell biology and development where its expression is tightly controlled (Laurenti et al., 2009). As such, many cancers have been suggested to derive from stem cells during development where Myc expression persists or is deregulated into the adulthood. Myc has various cellular functions ranging from proliferation, cell growth and differentiation to apoptosis. Thirty years after its discovery, the exact molecular mechanisms of Myc mediating these functions still remain elusive. As an immortalizing tool, however, Myc has been widely used to generate cell lines from different lineages, especially neural cell lines, some of which are still widely applied in neuroscience research.

Myc encodes a transcription factor that binds to E-box sequence CACGTG on genomic DNA. Myc protein dimerizes with another bHLH transcription factor Max through its bHLH/LZ domain, and its transregulatory domain can interact with numerous co-factors to execute its transcriptional activity on target genes including the transcription activator E2F-1 (Fig. 2). The critical function domains of Myc proteins have been mapped to their N- and C-terminus, which contain the transregulatory domain and the basic-helix-loop-helix-leucine zipper (bHLH/LZ) domain, respectively (Farina et al., 1992; Min et al., 1993; Min and Taparowsky, 1992).

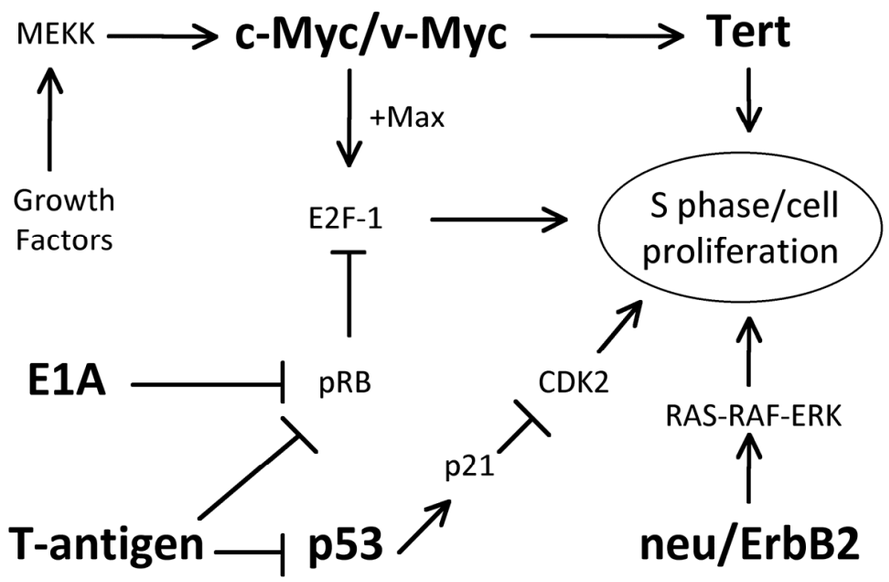


Fig. 2. Simplified cellular pathways involving immortalizing genes (labeled in bold).

V-Myc, the viral homologue of cellular Myc (c-Myc), was first discovered from a transforming retrovirus MC29 from chicken with spontaneous myelocytomatosis and subsequently cloned and sequenced (Alitalo et al., 1983; Reddy et al., 1983; Watson et al., 1983). V-Myc is expressed as a fusion protein Gag-Myc in MC29 and other related retroviruses. Although the Gag portion of the fusion protein seems to be dispensable in its transforming activity (Shaw et al., 1985), the first generation of v-Myc containing retrovirus for immortalization was created as fusion of v-Myc with part of Gag protein (Villa et al., 2000). Myc genes carry mutations, some of which potentiate their transforming activity. A frequently detected mutation Thr58 on c-Myc, which is equivalent to Thr61 on v-Myc, is often found in Burkitt's lyphomas where c-Myc was first discovered (Albert et al., 1994). A Thr58 to Ala substitution (T58A) in c-Myc has been found to promote its proliferative effect in NSCs, and a resulting NSC line immortalized by this mutant c-Myc exhibited enhanced cell proliferation compared with NSC line generated by the wild type gene (De Filippis et al., 2008).

Large T antigen is another popular immortalizing gene used to generate NSC lines. Numerous NSC lines have been derived by utilizing different forms of large T antigen (e.g. temperature-sensitive and N-terminally truncated mutants) from different regions of the developing CNS (Whittemore and Snyder, 1996). SV40 large T antigen is one of the early gene products during viral infection by polyomavirus SV40. SV40 is a double-stranded DNA virus first identified in 1960 and is responsible for formation of solid tumors upon infection. Large T antigen is involved in viral genome replication after infection and regulation of host cell cycle mainly through its perturbation of tumor suppressor protein p53 and the retinoblastoma protein (pRB) (Fig. 2), although several other cellular factors, including the transcriptional co-activators p300 and CBP, may also contribute to its transformation function (Ahuja et al., 2005).

p53 is a tumor suppressor protein and its function is involved in preventing cancer formation. P53 plays many cellular roles in its anti-cancer function, which include cell cycle regulation, apoptosis and genome stability. Upon activation, p53 can initiate a cell cycle arrest to allow DNA repair to take place, or signal cells to go on apoptosis if the DNA damage is unfixable. One mechanism of such function of p53 protein is that, upon activation, it can turn on the expression of p21, which forms complex with cyclin-dependent kinase 2 (CDK2) and inhibits its promoting activity in G1/S transition in the cell cycle (Wierod et al., 2008) (Fig. 2). Gene knockout of p53 or mutations that affect p53 binding to DNA results in unavailability of p21 to act as a stop signal in cell cycle. As such, cell will continue to divide and in some cases will form tumors. The inactivation of p53 can transform cells, and this also provides a way to immortalize cells. Indeed, NSPC clones have been reported to derive from p53 knockout mice (Tominaga et al., 2005; Yamada et al., 1999), but cautions have to be practiced in using these cells in transplantation since they are supposed to be sensitive to DNA mutations and therefore more likely to develop tumors in vivo.

E1A is one of the early gene products of adenoviruses and has also been reported to immortalize neural progenitor cells. The primary cellular target of E1A is pRB (Nevins, 1992). The tumor suppressor protein pRB prevents cell proliferation by inhibiting cell cycle progression. Dysfunction of pRB is often detected in many types of cancer. pRB is a member of pocket protein family and can bind and inhibit E2F-1 transcription activator, thereby preventing cell cycle from entering S phase. Upon adenoviral infection, E1A binds tumor

suppressor protein pRB (Fig. 2) and transforms cells with the help of another early gene product E1B, which binds p53.

The oncogene neu was first discovered in a neural tumor, and later was found to share the same sequence with human epidermal growth factor receptor 2 (HER2) and avian erythroblastosis oncogene B2 (ErbB2). HER2/neu is an oncogene that is involved in many types of cancer (Dougall et al., 1994). HER2/neu is a transmembrane receptor tyrosine kinase, and it clusters with other members of the EGFR family and initiates signal transduction pathways that lead to cell proliferation and differentiation. The downstream signaling pathways include the RAS-RAF-MAP kinase, the phosphatidyl inositol 3-kinase (PI3K), and the Akt pathways, where RAS-RAF-MAP kinase pathway plays a more important role to cell cycle progression, thus neural progenitor immortalization when HER2/neu is overexpressed (Fig. 2). Immortalization with neu has been reported (Frederiksen et al., 1988; Sherman et al., 1999), but rare.

Telomerase (Tert) is a reverse transcriptase that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3′-end of DNA strands in the telomere regions of chromosome. In nearly all dividing mammalian cells, telomere shortening is prevented by the activity of Tert, which ensures the sufficient DNA replication during cell division. The decreased expression level of Tert accompanied by telomere shortening of chromosome is often observed in cells that lose the capacity of cell division and become senescence. Overexpression of Tert often leads to cancer formation and also provides a tool for immortalization of neural progenitors (Bai et al., 2004; Roy et al., 2004; Schwob et al., 2008) (Table 1).

| **hNSPC clone** | **Tissue origin** | **Immortalizing gene** | **Reference** |
|---|---|---|---|
| B4, C2, C10 and others | 13 wks, brain | Tet-off v-Myc | (Sah et al., 1997) |
| HNSC.100 | 10-10.5 wks, diencephalic and telencephalic regions | v-Myc | (Villa et al., 2000) |
| hSC11V-TERT | 9-13 wks, spinal cord | hTERT | (Roy et al., 2004) |
| hNS2 | 10 wks, forebrain | v-Myc | (Villa et al., 2004) |
| hNPC-TERT | fetal SVZ (age?) | hTERT | (Bai et al., 2004) |
| CTX0E03 | first trimester, brain | c-MycER, conditional | (Pollock et al., 2006) |
| hc-NSC-F7b and others | 8-9 wks, cortex | v-Myc | (Cacci et al., 2007) |
| ReNcell VM | 10 wks, midbrain | v-Myc | (Donato et al., 2007) |
| ReNcell CX | 14 wks, cortex | c-Myc | (Donato et al., 2007) |
| T-IhNSC | 10.5 wks, diencephalic and telencephalic tissue | c-Myc (T58A) mutant | (De Filippis et al., 2008) |
| hVM1 | 10 wks, ventral mesencephalon | v-Myc | (Villa et al., 2009) |
| HB2.G2 | 11–14 wks, telencephalic tissue | Tet-on v-Myc | (Kim et al., 2011) |

Table 1. Summary of immortalized human NSPC clones.

### 2.2 Growth factor dependency

Genetic modification such as overexpressing an oncogene in a cell is expected to bypass growth factor stimulation and thus make possible the cell cycle progression. In reality, however, many neural progenitor cell lines generated by oncogenes mentioned above are heavily dependent on growth factors for their in vitro proliferation. Upon removal of these growth factors, progenitor cells cease proliferation and go on with differentiation towards mature cell types in correlation with the drastic down-regulation of the oncogene expression (such as in the case of v-Myc). Although the mechanism of this phenomenon (oncogene down-regulation upon growth factor withdrawal) is still unclear (perhaps some type of feedback loop system is controlling the exogenous oncogene expression), it provides an extremely important safety prerequisite in using these immortalized cells especially in a transplantation scenario, since the primary phenotypes of a tumorigenetic cell are growth factor independency and loss of contact-inhibition.

Although some of the early generated neural progenitor lines were propagated in mediums containing fetal calf serum (FCS) and sometimes in combination with growth factors, more defined serum-free medium has been widely applied in culturing these cells and FCS has been used as inducing reagent for differentiation into certain neural cell types such as astroglia. Growth factors such as fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) have been shown to promote proliferation of immortalized neural progenitors (Kitchens et al., 1994) and are commonly used in serum-free medium as mitogens to promote neural progenitor cell division. In combination of growth factor stimulation and immortalizing genes, NSCs can be expanded in large quantity without obvious phenotypic abnormalities. Then, upon growth factor withdrawal, NSCs simultaneously differentiate along distinct cell lineages. Differentiation inducing factors such as cytokines and neurotrophic factors can be applied to facilitate differentiation and survival of certain cell types. For example, neurotrophin 3 (NT3) and brain-derived neurotrophic factor (BDNF) induce neuronal differentiation and promote neurite outgrowth, while bone morphogenic proteins (BMPs) and leukymia inhibitory factor (LIF) induce astroglial differentiation by upregulating expression of astrocytic marker, glial fibrillary acidic protein (GFAP). Sonic hedgehog homolog (SHH) and platelet-derived growth factor (PDGF) are mitogens to subpopulations of neural progenitors and favor differentiation towards oligodendrocyte lineage.

## 3. Neural stem/progenitor cell clones as in vitro models

As in vitro models, immortalized neural progenitor clones possess advantages over primary cells isolated from tissue. Firstly, one can produce large quantity of immortalized cells because of the activity of exogenously introduced oncogenes that keep cell proliferation almost infinitely in the presence of appropriate growth factors. Secondly, in vitro expanded immortalized progenitors have cellular homogeneity of higher degree than primary cells since most of these progenitor clones reported are clonal. Lastly, probably because of their promoted cell cycle properties, immortalized progenitor clones are more assessable to genetic modification and more likely to maintain gene expression over passages. There are excellent reviews in the past categorizing most of these clones and describing scientific insights resulted from them (Martinez-Serrano and Bjorklund, 1997; Vescovi and Snyder, 1999; Whittemore and Snyder, 1996). In this section, we will review more recent works on

these clones and new clones generated from our lab and others, and focus on their value as in vitro models.

### 3.1 Models of neural progenitor development

Neural progenitors exhibit spatiotemporal diversity during CNS development (Li and Shi, 2010). To model neural progenitor development, immortalized clones have been isolated from different regions of the CNS at different developmental stages (Whittemore and Snyder, 1996).

Hippocampus plays pivotal roles in learning and memory, and is one of the few sites in adult CNS that retain the capacity of neurogenesis. Therefore, the proliferation and differentiation of hippocampal neural progenitor are of great interests to neurobiologists. HiB5 was isolated as a hippocampal neuronal progenitor from E16 rat (Renfranz et al., 1991), and has been used to model hippocampal neurogenesis. It was found that activation of RARgamma promotes proliferation of HiB5 cells in vitro (Chung et al., 2000). When HiB5 cells were treated with all-trans- or 9-cis-retinoic acid (RA), a significantly increased proportion of these cells were found in S-phase. This was accompanied by increased level of bcl-2 mRNA, while the level of bax mRNA was not affected, which suggests that retinoid treatment increases viable cells by enhancing proliferation rather than suppressing cell death. Furthermore, the proliferation promoting effect of retinoid on HiB5 cells can be mimicked by RARgamma-selective agonist and blocked by its antagonist (Chung et al., 2000). HiB5 cells can model differentiation of hippocampal neurons as well. To give an example, PKA signalling pathway was examined for its role in hippocampal neuronal differentiation process. Kim G et al. demonstrated that treatment of HiB5 cells by cyclic AMP (cAMP) in a serum-free medium containing N2 supplement induced drastic neurite outgrowth of these cells and inhibition of their proliferation (Kim et al., 2002). Phosphorylation of cAMP responsive element binding protein (CREB) was observed accompanied by increased expression of neuronal markers including neurofiliments and decreased expression of glial markers such as nestin and GFAP. Furthermore, overexpression of a GFP-fused protein kinase A (PKA) catalytic subunit alpha protein induced neurite outgrowth in HiB5 cells. Altogether, the authors demonstrated the critical involvement of PKA pathway in hippocampal neuronal differentiation using HiB5 clone as an in vitro model. More recently, another Korean group applied a traditional Korean medicine, Scutellaria baicalensis extract, in the culture of HiB5 cells and showed that it enhanced cell survival of HiB5 and increased their differentiation into choline acetyltransferase (ChAT) positive cholinergic neurons. Along with in vivo data, the authors suggested that Scutellaria baicalensis extract might be used as a neuroprotective medicine in cerebral ischemia (Heo et al., 2009).

Hippocampus maintains on-going neurogenesis in the adult CNS, and yet many of the newborn hippocampal neurons die shortly after birth especially when brain insults occur. Using HiB5 as a model cell, Cacci E et al. were able to show that increased cytokine release by activated microglia may contribute to the death of hippocampal neurons (Cacci et al., 2005). When TNFalpha, as well as conditioned medium from activated microglia, was added to the culture medium, HiB5 cells quickly ceased proliferation and underwent significant cell death. Both HiB5 and microglia express TNF receptors, TNF-R1 and TNF-R2, which may mediate this effect. Hippocampus is vulnerable to different types of insults that

lead to neuronal cell death in this brain region. Glucocorticoid (GC), a steroid hormone, participates in normal glucose metabolism. However, when high hippocampal GC level is prolonged, for example in prolonged stressed condition, hippocampal neurons undergo apoptosis. Heat shock protein Hsp27 has been shown to antagonize GC-evoked apoptosis in HiB5 cells (Son et al., 2005). When HiB5 cells were treated with dexamethasone (DEX), a synthetic GC, apoptosis occurred. Interestingly, expression of Hsp27 was also induced upon this treatment. To evaluate possible function of Hsp27 in this process, Son GH et al. overexpressed several constructs in HiB5 cells before DEX treatment and demonstrated that Hsp27 protects hippocampal neurons from GC-induced apoptosis (Son et al., 2005). The same research group also demonstrated that another heat shock protein Hsp25 is involved in neuroprotective effect in hippocampal neurons as well. Furthermore, phosphorylation of Hsp25 mediated by MAPK and ERK signalling is important for its translocation from cytoplasma to nucleus, where it protects nuclear structure, thereby preventing neuronal cell death (Geum et al., 2002). P62, a ubiquitously expressed phosphoprotein, is implied to play a role in protecting hippocampal neuronal survival. When overexpressed in HiB5 cells, p62 not only reduces cell death, but also promotes neuronal differentiation of the cells (Joung et al., 2005). In addition, pre-treatment by vitamin D3 substantially reduced the degree of DEX-induced apoptosis in HiB5 and primary hippocampal neurons suggesting a cross-talk between vitamin D3 and GC pathways (Obradovic et al., 2006).

Isolated from rat E14 striatum, neural progenitor clone ST14A has been used as a model for striatum-derived neurons (Cattaneo and Conti, 1998). The availability of large number of cells made it possible and convenient to examine signalling pathways in these cells. Wnt signalling has been shown to involve in NSC differentiation by expressing necessary Wnt receptors (Lange et al., 2006). ST14A cells express JAK/STAT signalling components and are susceptible to cytokine stimulation leading to cell proliferation (Cattaneo et al., 1996). Ventrally born neurons such as cortical interneurons reach neocortex by tangential migration. The migratory property of ST14A has been realized and used as a model of neuronal migration in vitro. Hepatocyte growth factor /scatter factor (HGF/SF) has been involved in migration and proliferation in many types of epithelial cells. HGF/SF and its receptor Met are also present in the developing CNS as well as ST14A cells. When ST14A cells were exposed to HGF/SF in culture, the cells quickly changed morphology and increased cell motility, a process that involves PI3-K pathway as revealed by pharmacological blocking analysis (Cacci et al., 2003). The cytoskeletal rearrangement including actin network and dissociation of beta-catenin from N-cadherin were also observed in ST14A cells upon treatment of HGF/SF, but not nerve growth factor (NGF), BDNF, NT3 and ciliary neurotrophic factor (CNTF) (Soldati et al., 2008). ErbB family proteins play important function in neuronal migration. Gambarotta et al. demonstrated that ErbB4, but not ErbB1-3, is a crucial receptor of neuregulin1 (Nrg1) in activating migration of ST14A cells (Gambarotta et al., 2004). By gene expression profiling analysis, the same group subsequently identified the epidermal growth factor receptor pathway substrate 8 (Eps8), a multimodular regulator of actin dynamics, as a key mediator of Nrg1/ErbB4 induced neuronal migration (Fregnan et al., 2011).

Radial glia (RG) is a transient cell type during CNS development. It is known by their unique bipolar radial morphology, which is important for its function of supporting neuronal migration. Later, RG has been shown to give rise to neurons and probably the major neuronal precursors throughout the developing CNS (Noctor et al., 2002). We

generated first RG clones by v-myc immortalization and demonstrated their properties in vitro including expression of RG specific markers and ability to support neuronal migration. Because of their transient nature, isolation and propagation of RG in vitro have been unsuccessful. The v-myc immortalized RG clones (RG3.6 and L2.3) showed high proliferation rate in the presence of FGF2 and maintained RG markers over many passages, which provides an in vitro model system to allow examination and manipulation on this cell type (Hasegawa et al., 2005; Li et al., 2004). We did observe a gradual change in expression of certain markers in cultured RG clones during passage. For example, brain lipid binding protein (BLBP), a specific RG marker, decreases while a marker for GRPs, A2B5, increases. This initial observation eventually led us to discover a transition from RG to restricted precursors during embryonic forebrain development (Li et al., 2004; Li and Grumet, 2007). To further stablize RG in culture, we introduced actived form of Notch1 into clone L2.3 by retroviral infection. We found that active Notch1 signalling inhibited GRP marker expression and enhanced RG morphology and gene expression (Li et al., 2008a).

Among series of neural progenitor clones generated in our lab, clone L2.2 showed neuronal restricted differentiation in vitro. Moreover, L2.2 gives rise to exclusively GABAergic neuronal subtype upon FGF2 withdrawal as evaluated by their expression of TuJ1, GADs, Dlxs and calretinin (Li et al., 2008b). Neurons derived from L2.2 fire action potential in culture, and this functional differentiation is accelerated when cocultured with RG clone RG3.6 probably through a cell-cell contact mechanism, because the conditioned medium from RG3.6 was not able to exert the same effect. The neuronal progeny of RG clone identified by TuJ1 positivity exhibits projection neuron phenotypes, e.g., bipolar simple morphology, and the majority (87.4 ± 1.5%) of which are glutamate immunoreactive. Based on our gene expression analysis, L2.3 differentiated culture also expressed T-brain-1 (Tbr-1) transcription factor, which, along with Pax-6 and Tbr-2, is an essential marker for projection neuron differentiation in vivo (Hevner, 2006). During cortical neurogenesis, most, if not all, of glutamatergic projection neurons come from RG, which later differentiate into glial cells. Therefore, clone L2.2 and L2.3 (or RG3.6) generate interneurons and projection neurons, respectively in culture, and they may serve as in vitro models to study interaction between these different neuronal subtypes during cortical development.

### 3.2 Gene profiling and high-throughput analysis

The availability of large quantity of cells by in vitro expansion of immortalized neural progenitors made large-scale analysis possible. For example, gene expression profiling experiment using ST14A cells overexpressing GDNF has revealed upregulated genes that are involved in neural differentiation and migration (Pahnke et al., 2004). ST14A cells overexpressing CNTF demonstrated increased proliferation, metabolic activity and resistance to stress during early differentiation (Weinelt et al., 2003). Similarly, gene expression profiling analysis confirmed this observation by showing upregulated genes that are involved in stress response pathway of this CNTF-ST14A cells (Bottcher et al., 2003). By overexpressing activated Notch1 gene in RG clone L2.3, we generated a new clone NL2.3 that exhibits enhanced RG marker expression and exaggerated RG morphology (Li et al., 2008a). To explore genes that are responsible for RG phenotype, we conducted gene expression comparison between NL2.3 and its parental clone L2.3. As expected, RG related genes such as BLBP, nestin, tenasin and vimentin were upregulated in NL2.3, and surprisingly we also found that cell adhesion molecules, especially nidogen1 (showing 50

fold increase comparing to L2.3), were upregulated, which may explain the better attachment of NL2.3 cells on laminin-coated substrate. We further confirmed the functional role of nidogen1 in mediating cell adhesion by antibody blocking experiments, and revealed a previously unrecognized link between Notch1 signalling and cell adhesion. In addition, we showed that primary RG cells also express nidogen1 in a secreted fashion demonstrating the physiological significance of this result (Li et al., 2008a).

Proteomics in NSCs is still in its early stage. Nevertheless, large quantity of cells from immortalized progenitors is well suited for this type of analysis. Clone ST14A and an immortalized human NSC clone ReNcell VM have been applied in 2-DE proteomic profiling leading to meaningful discoveries (Beyer et al., 2007; Hoffrogge et al., 2007). Another significant application of expandable neural progenitor cells is high throughput (HTP) screening. Beside drug screening in neural progenitors in pharmaceutical industry, there is also an increasing demand for HTP protocol for genetic analysis on a genome scale. Park JY et al. developed two HTP-optimized expression vector systems that allow generation of red fluorescent protein (RFP)-tagged target proteins (Park et al., 2007). Using these systems, the authors screened sixty representative human C2 domains for their neuronal promoting effect, and identified two C2 domains for their further study. This is another good example for taking advantages of large quantity of cells from immortalized NSC clones.

### 3.3 Models for neurological diseases

NSC clones are capable not only to facilitate research on NSC differentiation, but also provide model systems for neurological diseases. Here, we give an example on clone ST14A. Derived from embryonic striatum, ST14A cells have been used as in vitro model for Huntingtin's disease (HD). In fact, it has been shown that under serum-free condition, ST14A cells were able to differentiate into DARPP-32-positive medium spiny neurons spontaneously and displayed electrophysiological properties similar to those of medium spiny neurons (Ehrlich et al., 2001). Protein aggregation is the hallmark of neurodegenerative diseases including HD. Ossato G et al. developed a so-called number and brightness method to monitor aggregation of Huntingtin exon 1 protein directly in live ST14A cells and found that the mutant protein underwent a two-step aggregation process, an initial phase of monomer accumulation and oligomer formation followed by protein inclusion depleting monomers in the cytoplasma (Ossato et al., 2010). The pathology of HD has been well documented, and yet its underlying molecular mechanisms still remain elusive. Using the same clone, Sadri-Vakili G et al. demonstrated that epigenetic regulation plays a role in HD progression (Sadri-Vakili et al., 2007). It was found that despite no change in overall acetylated histone levels, histone H3 was hypo-acetylated at the promoter regions of certain down-regulated genes in ST14A cells as well as in R6/2 mice, an animal model for HD. Furthermore, histone deacetylase (HDAC) inhibitor treatment increased level of acetylated histones and seemed to correct expression of misregulated genes, suggesting a potential therapeutic application of HDAC inhibitors for HD (Sadri-Vakili et al., 2007). Altered cholesterol biosynthetic pathway has been reported to involve in HD. The expression level of several key genes in the cholesterol pathway is severely disrupted in brain tissues of HD mice and human patients (Valenza et al., 2005). Mutant Huntingtin was introduced into ST14A cells and it significantly reduced total cellular cholesterol mass. By adding cholesterol back to the cells, the authors were able to prevent cell death of mutant ST14A in a dose-dependent manner. This report uncovered the cholesterol pathway as a

novel player in HD, which could be used as a potential target for HD treatments. Phosphorylation of Huntingtin protein appears to be protective in HD, which is mediated by phosphatase calcineurin and phosphokinase Akt. Regulator of calcineurin (RCAN1-1L) is suppressed in HD patient samples, and overexpression of RCAN1-1L in ST14A cells that contain mutant Huntingtin gene increases phophorylation of Huntingtin and reduces ST14A cell death (Ermak et al., 2009). Therefore, the authors claim that RCAN1-1L might be a mediator for HD progression and offer an alternative avenue for drug treatments.

### 3.4 Testing biomaterials

Biomaterial science has been a fast-growing field providing promising materials for tissue engineering. Biocompatibility of these materials is a big issue since they will be in contact with human cells and tissue. Large quantity of cells, especially cells derived from immortalized neural progenitors are well suited to test toxicity of biomaterials to cells in culture, because in many cases, transplantation of neural progenitor cells is accompanied by biomaterials in the hope that the latter can potentiate stem cell differentiation and migration. The biocompatibility of a variety of biomaterials including polymers and nanofibers were tested using immortalized progenitor cells as first step towards biological applications. Poly(lactic-co-glycolic acid) (PLGA) was compared with other types of polymers in culturing with clone HiB5, where it performed the best in terms of supporting cell viability and neurite outgrowth. This result provided evidence that PLGA could be used as a scaffold for NSC transplantation for nerve regeneration (Bhang et al., 2007). Polymers can also be created to have different patterns and dimensions by various means. Electrospun poly(l-lactide) (PLLA) fibers with different parameters were tested in culture with NSPC clone C17.2. The cells displayed significantly different growth and differentiation depending on fiber pattern and dimension they adhered (He et al., 2010). PLLA can also be modified by tethering laminin-deirved peptides through a cross-linking reagent, and the modified PLLA showed significant improvement in supporting cell survival and neurite outgrowth of C17.2 cells (He et al., 2009). A UV pre-irradiation followed by UV grafting technique can create gradients of carboxyl group on poly(acrylic acid) (PAA) substrates. It was shown that C17.2 cells adhered to these substrates and appeared to respond the carboxyl gradient by directionally sending out neurites (Li et al., 2005). Polymers modified to be electronic affected seeding density of C17.2 cells and may have effects on stem cell differentiation as well (Salto et al., 2008). This report provided an alternative electronic control over NSC differentiation.

Nanotechnology has revolutionized the biomaterial field. Polymers and scaffolds produced at nano-scale have proven to be superior than other material in biological applications. Biomaterials made by nanopolymers possess high surface-to-volume ratio and offer a variety of topographic features that may promote cellular behavior. Nanofibrous scaffolds fabricated with different ratios of poly(epsilon-caprolactone) (PCL) and gelatin were tested in their ability to promote neuronal differentiation of C17.2 cells, and the PCL/gelatin 70:30 ratio generated the best biomaterial suited for nerve regeneration (Ghasemi-Mobarakeh et al., 2008). Similar cell culture tests were also performed on electroconductive polymeric nanowire templates showing that polypyrrole coating improved their effects on cell adhesion and proliferation (Bechara et al., 2011).

## 4. Neural stem/progenitor cell clones for transplantation

The promise for stem cells including NSCs is someday they may be used as therapeutic cures for diseases. In animal models, there are numerous reports that support this promise and demonstrate great potential of these cells in tissue protection, replacing lost cells and restoring behavioural function when transplanted in a variety of diseases and trauma. Immortalized NSCs provide unlimited cell number and maintain stem cell characteristics such as differentiation potentials to certain cell lineages, and therefore are outstanding candidates for this purpose. Attempts have been made for NSCs to go into human patients hoping they behave similarly to transplanted cells in animals. However, the major concern is the safety of these cells. NSCs derived from either primary tissue or immortalized counterparts have tendency to form tumors upon transplantation since their cell cycle dynamics have been altered by stimulation of growth factors and/or exogenous oncogenes in the case of immortalized NSCs. Although researchers have claimed that some of the oncogenes are non-transforming (e.g. Myc) and their expression is drastically reduced after NSC differentiation and transplantation in vivo, precautionary measures have to be taken into practice to ensure the safety of these cells in clinical settings. In this section, we will review up-to-date reports from others' work on transplantation using immortalized neural progenitor clones in normal and diseased CNS. We will also describe our work using neural progenitor clones to treat spinal cord injury in rat. Finally, we will touch on tumor inhibition, an unexpected property of NSCs revealed by studies using immortalized NSPC clones.

### 4.1 Transplantation into normal CNS

In order for NSCs to fulfil neural reparative goal, a key property is to be able to differentiate into mature cell types in regions of transplantation. Neurogenesis and neuronal differentiation are already completed in most regions of the adult brain. Therefore, it is a bit of challenge for transplanted cells to differentiate under this "mature" environment. Surprisingly, however, permissive cues must be still present in neonatal and adult CNS allowing neuronal differentiation to occur. Immortalized NSCs have been transplanted first into normal animals and tested in their survival, migration and differentiation potentials under in vivo environments. For example, RN33B, a conditionally immortalized neural progenitor clone derived from E13 rat medullary raphe nucleus (Whittemore and White, 1993), was transplanted into various regions of neonatal and adult brains. In cerebral cortex and hippocampus formation, RN33B cells survived up to 24 weeks and differentiate with morphologies similar to pyramidal neurons, granule neurons and polymorphic neurons in a region-specific manner (Shihabuddin et al., 1995). Electron microscopy and immunohistochemistry demonstrated that differentiated RN33B cells received synapses from host neurons. In striatum, transplanted RN33B cells survived, integrated and differentiated into neurons and glia, some of which displayed morphological and phenotypic properties of medium-sized spiny neurons. These neurons were also found to form connections with primary striatal target, the globus pallidus, by retrograde tracing analysis (Lundberg et al., 1996). Furthermore, GFP-labelled RN33B cells transplanted into hippocampus exhibited remarkable neuronal morphology and were capable of firing action potentials and receiving synaptic inputs of both excitatory and inhibitory nature (Englund et al., 2002). In contrast to neuronal differentiation mentioned above, RN33B cells transplanted

in the mesencephalon predominantly differentiated into astroglia (Lundberg et al., 2002). These observations suggest that regional cues are still present in the adult brain that can direct differentiation of transplanted neural progenitors into certain cell types, which can integrate into local tissue structures, although it has been shown that neonatal brains have greater capacity to encourage differentiation, especially neuronal differentiation, of transplanted cells than adult brains suggesting a decline of permissive cues during CNS development (Shihabuddin et al., 1995).

On the other hand, immortalized progenitors derived from one region of the brain can differentiate into cell types specific to the other indicating plasticity of these cells that may broaden their reparative application in different areas of the CNS. Indeed, when RN33B, along with another NSPC clone C17.2 derived from postnatal cerebellum, was transplanted into adult retina, they survived up to 4 weeks and differentiate into both neurons and glia in all major retinal cell layers including retinal pigment epithelium (Warfvinge et al., 2001). Intrinsic molecular natures of individual progenitor clones have to be considered when applying them into transplantation therapies. They all may be multipotent in culture, but have limited potentials to differentiate in vivo. For example, two weeks after transplantation into adult striatum, unlike RN33B cells to become both neurons and glia, clones HiB5 and ST14A differentiate mostly into glial cells (Lundberg et al., 1996), indicating their distinct intrinsic properties including possible distinct responsiveness to the local host environment. Worthy of noting is that these NSC-derived astroglia functionally integrated into host by showing reactive phenotype in response to brain damage (Lundberg and Bjorklund, 1996). By using immortalized NSC clones, the above mentioned results demonstrated the plasticity of NSCs to differentiate into distinct cell types by responding to local cues, and the fact that adult brains still retain, to a certain degree, the ability to direct differentiation of exogenous neural progenitor cells. C17.2 cells were also transplanted into lumbar region of normal spinal cord where they differentiated into nonmyelinating ensheathing cells. In addition, they appeared to induce host axons to form de novo tracts aiming at graft site probably through the action of their secreted neurotrophic factors, suggesting NSCs may also trigger regenerative potentials of host tissue needing for repair (Yan et al., 2004).

### 4.2 Neurodegenerative diseases

Neurodegenerative diseases are caused by region-specific, progressive cell death and include Parkinson's disease (PD), HD, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). This is an area where cell replacement therapies are on high demand. We will take PD as an example to highlight the value of immortalized progenitors in testing effects of NSCs in PD animal models in terms of neuroprotection and differentiation into desired neuronal cell type. The common pathology of PD patients is the progressive loss of dopaminergic neurons in the substantial nigra that normally project axons onto striatum. As a result, dopamine level in the striatum greatly reduced, which is believed to induce PD symptoms such as dystonic cramps and dementia (Weidong et al., 2009). Attempts have been made to restore dopamine level by various means, which seem to alleviate symptoms to a certain degree, but they can not prevent PD progression and replenish the lost neurons.

Clone C17.2 has been transplanted into normal and lesioned striatum of the adult rat brain in testing their behaviour in local environment where dopaminergic neurons reside. Consistent with the notion mentioned above, these cells were able to turn on neuronal markers and tyrosine hydroxylase (TH), an enzyme critical to dopamine systhesis, suggesting that local cues are still present and powerful enough to direct multipotent immortalized NSCs to differentiate into local neuronal subtypes (Yang et al., 2002). Efforts have also been made to reinforce dopaminergic differentiation of transplanted progenitors to maximize their effects in PD animal models. For example, C17.2 cells have been genetically modified to overexpress enzymes (e.g. TH and GTP cyclohydrolase 1) (Ryu et al., 2005), Nuclear receptor related 1 protein (NURR1) (Li et al., 2007) and secreted factors (e.g. neurturin) (Liu et al., 2007) towards the goal of getting more dopaminergic neurons. Both TH and GTP cyclohydrolase 1 are required for efficient L-DOPA synthesis, since HPLC assays demonstrated that L-DOPA released from C17.2 cells that are transduced with both genes (C17.2-THGC) is 760-fold higher than that from C17.2 cells transduced with TH gene alone (C17.2-TH). Following transplantation into striatum of PD rats, C17.2-THGC was able to promote animals' behavioural improvement when compared with transplants with parental C17.2 cells (Ryu et al., 2005). NURR1 has been shown to induce downstream target genes such as TH and facilitate dopaminergic differentiation. When overexpressed in C17.2 cells, NURR1 enhanced cell differentiation into dopaminergic neurons and even promoted behavioural recovery to a certain extent (Li et al., 2007). Unlike in normal CNS, transplanted neural progenitors have to overcome unfavourable environment in diseased tissue to survive and differentiate. Therefore, manipulations that mitigate local environment may prove to facilitate beneficial outcomes. Neurturin is a secreted factor that belongs to glial cell-derived neurotrophic factor (GDNF) family. Like GDNF, neurturin exerted neuroprotective effects on host dopaminergic neurons after transplantation into lesioned striatum. In addition, neurturin also promoted dopaminergic differentiation of C17.2 cells when overexpressed, and animal behaviour as well (Liu et al., 2007). Similarly, interleukin-10 overexpression in C17.2 lowered immune response of host tissue to create a beneficial microenvironment for cell survival and differentiation (Wang et al., 2007). In addition, treatment of melatonin in combination with C17.2 showed neuroprotection in PD models (Sharma et al., 2007).

### 4.3 Brain and spinal cord injury

Traumatic brain injury (TBI), also called acquired brain injury or simply head injury, occurs when a sudden trauma causes damage to the brain. TBI can result when the head suddenly and violently hits an object, or when an object pierces the skull and enters brain tissue. Symptoms of a TBI can be mild, moderate, or severe, depending on the extent of the damage to the brain. Besides direct damage of brain tissue by trauma, secondary damage in TBI results from toxic effects of a variety of modulators that magnify the initial traumatic damage. Among these modulators are the excitatory transmitter glutamate, the intracellular messenger calcium, and the intercellular messenger nitric oxide. Glutamate-induced toxicity, also called excitotoxicity, occurs from excess glutamate release following trauma. Because of the very limited capacity of the brain for self-repair, cellular transplantation has been explored to improve repair. The goal of many studies has been to replace the lost

neurons (Bjorklund, 2000; Gage, 2000; Lie et al., 2004; McKay, 1997). Although instructive cues for neuronal differentiation in normal adult brains seem still exist (albeit declining comparing to neonatal brains), the local environment around the injured brain region could be very different. Transplanted cells have to overcome extra hurdles such as glutamte excitotoxicity and high concentration of released cytokines to survive and differentiate. Nevertheless, studies in the past have shown that immortalized progenitors can survive and differentiate upon transplantation in TBI. C17.2 cells were injected into adult mouse brains at 3 days after lateral controlled cortical impact injury (Riess et al., 2002). The study demonstrated cell survival of C17.2 as long as 13 weeks post-transplantation and significant improvement in motor behavior of C17.2 transpalnted animals as compared with those received human embryonic kidney cells. It was also found in this study that C17.2 cells implanted contralateral to the impact side differentiated into mostly neurons, while the cells implanted ipsilateral to the impact side differentiated into astroglial cells as well as neurons consistent with the notion that injured environment favors glial differentiation of transplanted NSCs (Riess et al., 2002). In order to improve the efficacy of NSC mediated beneficial effects in TBI treatemnt, immortalized progenitors engineered to express neurotrophic factors have been utilized. As expected, these genetically modified cells showed neuroprotective effects and improved functional recovery of the animals. For example, C17.2 overexpressing GDNF (GDNF-C17.2) improved survival of transplanted cells, enhanced neuronal differentiation of these cells and promoted learning behavior of the TBI rats at 6 weeks after transplantation comparing to parental C17.2 cells (Bakshi et al., 2006). Similarly, HiB5 progenitor clone engineered to secrete NGF (NGF-HiB5), when transplanted peripheral to the TBI site, decreased apoptosis of the host hippocampal neurons and improved motor and cognitive function comparing to controls (Philips et al., 2001).

Spinal cord injury (SCI) is a severe CNS injury often resulting in long-term disability. Immediately after contusion there is limited histological evidence of damage followed by neuronal death (hours), and that is followed by macrophage infiltration, Wallerian degeneration and astrogliosis (days-weeks). Similarly to TBI, physical disruption of spinal cord causes membrane depolarization and results in massive glutamate release, which is not only excitotoxic to injured cells themselves but surrounding cells. Several weeks after contusion injury in humans as well as in rats (but not in mice), cystic cavities develop surrounded by gliotic scars associated with extracellular matrix including chondroitin sulfate proteoglycans (CSPG), which is not hospitable to axonal regeneration (Busch and Silver, 2007).

Advances in cell characterization and isolation are opening new opportunities for cell transplantation to repair tissue damage by replacing cells that restore lost function (Gage, 2000). Numerous immortalized NSCs have been implanted into SCI to test their differentiation potentials and efficacy on functional recovery. Embryonic raphe nucleus-derived progenitor clone RN33B morphologically differentiated into multipolar neurons resembling nearby endogenous ones when transplanted into gray matter of normal spinal cord. However, only relatively undifferentiated RN33B cells with bipolar morphology could be found at 2 weeks after transplantation into rat spinal cords with various types of injuries with depletion of endogenous neurons due to the lesions. The authors suggested that cell-cell contact mechanisms contribute to instructive local cues for permissive neuronal differentiation of transplanted progenitors, and that molecules released from the injury site

may also have prevented these cells from becoming neurons (Onifer et al., 1997; Whittemore, 1999). On the other hand, implanted neural progenitors secrete neurotrophic factors themselves, which may alter the microenvironment they encounter inside the CNS tissue. Clone C17.2 cells have been shown to naturally express and secrete several trophic factors including NGF, BDNF and GDNF both in vitro and in vivo after transplantation (Lu et al., 2003). When implanted into adult rat spinal cords with cystic dorsal column lesion, C17.2 cells promoted extensive growth of endogenous axons. Elevated expression of one factor NT3 by genetic modification in C17.2 expanded this promoting effect (Lu et al., 2003), and improved C17.2 cell survival near the lesion site and functional recovery analyzed by Basso-Beattie-Bresnahan (BBB) scoring (Zhang et al., 2007). We reported that an immortalized neural progenitor clone sharing properties with NSPC and radial glia (RG3.6) migrated extensively in the injured rat spinal cord and improved open field walking when transplanted acutely following contusive SCI (Hasegawa et al., 2005). The transplanted RG3.6 cells partially protected the rat spinal cord against several aspects of secondary injury including loss of axons and myelin as well as accumulation of CSPG and macrophages (Hasegawa et al., 2005).

Patients with SCI not only lose motor function below the injury site, but often develop debilitating neuropathic pain (allodynia) over time (Siddall et al., 1999), which diminishes the quality of their lives. The mechanisms underlying allodynia may be very complex and many possible factors contribute to these symptoms (Hulsebosch, 2005). One direct factor is the loss or reduction of inhibitory tone in the spinal cord sensory processing due to injury. Therapeutic strategies that prevent induction of allodynia, such as cell transplants that release anti-nociceptive substances, can be used to enhance the endogenous descending inhibitory neurotransmitter systems, such as GABA and serotonin (5HT). Immortalized progenitor RN33B cells were therefore used as a vehicle to deliver GABA in SCI. RN33B cells overexpressing GAD67, an enzyme critical for GABA synthesis, were transplanted into lumbar subarachnoid space of the rat spinal cord with chronic constraint injury (CCI) (Eaton et al., 1999b). Seven weeks after transplantation, RN33B cells were found on the pial surface of the spinal cord, and the animals that received these cells showed significantly reduction of both tactile and temperature allodynia comparing to those that received control cells. In the same CCI injury model, RN33B cells overexpressing BDNF or galanin have also been shown to have beneficial effects in reducing allodynia (Cejas et al., 2000; Eaton et al., 1999a). When C17.2 NSCs were transplanted into injured spinal cord, they primarily differentiated into astrocytes, which may result in sprouting of dorsal horn nocioceptive neurons and in turn allodynia of the animals. GDNF, when overexpressed in transplanted C17.2 cells, reduced nocioceptive fiber sprouting and allodynia to a certain extent suggesting a protective or analgesic effect of GDNF on injury-induced neuropathic pain (Macias et al., 2006). We have isolated cortical GABAergic interneuron progenitor clones from rat embryonic forebrains and demonstrated their restricted interneuronal differentiation in culture. We are further testing these intrinsic GABAergic progenitor clones in vitro and after transplanation in SCI, hoping that the cells can differentiate into neurons with GABAergic phenotype in SCI, not only to release GABA to reduce allodynia, but also to integrate into local neuronal circuitry and permanently eliminate the pain-like syndrome. Towards that goal, we have demonstrated that one such clone exhibited spontaneous synaptic activity when cocultured with E17 hippocampal neurons (Li et al., 2011).

### 4.4 Tumor inhibition

One surprising and yet interesting feature of NSCs when implanted in vivo is their ability to target tumorous tissues and inhibit their growth. For example, immortalized neural progenitor clones isolated from different regions of the embryonic brain, HiB5 (hippocampus) and ST14A (striatum primordium), were transplanted into nucleus Caudatus of Fisher rats along with N29 glioma cells. Both progenitors exhibited anti-tumor activity and prolonged animal's survival. Clone HiB5 was also shown to inhibit an additional tumor type and even be effective when transplanted 1 week after tumor cells inoculation (Staflin et al., 2004). Clone C17.2 was also tested in their tumor-tropic capacities in a similar paradigm, and the results showed that these cells were able to inhibit tumors of both neural and nonneural origin (Brown et al., 2003). Furthermore, C17.2 cells were able to migrate into the tumor mass even when injected via peripheral vasculatures showing great homing capacity of these cells that can be used to deliver therapeutic agents. The mechanism of the NSC homing phenomena towards tumor is not very clear. C17.2 chemotactic migration was tested in vitro by conditioned medium prepared from glioma culture as well as 13 different tumor-associated growth factors (Heese et al., 2005). The results showed that scatter factor/hepatocyte growth factor (SF/HGF) was the most potent one in attracting C17.2 cells in culture. In addition, antibody against SF/HGF was able to block the migratory behaviour of these cells stimulated by glioma-conditioned medium. Furthermore, Allport JR et al. showed tumor-targeting acivity of C17.2 in vivo and identified two other factors that are involved in this NSC homing event (Allport et al., 2004). This study showed that C17.2 cells that were transduced to express luciferase (C17.2-luc) accumulated onto tumors in mice carrying Lewis lung carcinomas. In vitro analysis showed that accumulation of C17.2-luc cells on tumor-derived endothelium (TEC) can be inhibited by functional blocking antibodies against SDF-1alpha and CD49d suggesting the involvement of SDF-1alpha/CXCR4 receptor and alpha4-integrin in the recruitment of C17.2-luc cells (Allport et al., 2004).

Unlike C17.2, HiB5 cells have not been able to show homing capacity towards tumors, even though HiB5 cells exhibited growth-inhibitory effect when they were cotransplanted with tumor cells into animals. Honeth et al. introduced the chemokine receptor CXCR3 to HiB5 cells and demonstrated its functionality by responding to ligand stimulation and activating downstream signaling pathways such as ERK and SAPK/JNK (Honeth et al., 2006). Upon transplantation, these modified cells showed enhanced migration towards glioma that expressed CXCR3 ligands, IP-10 and I-TAC, in comparison with parental HiB5 cells. This study provided proof-of-concepts that immortalized progenitors can be genetically modified and acquire homing capacity towards tumor to either inhibit tumor growth on its own or deliver therapeutic agents for local treatments. Among good examples of this notion is the study that was carried out by Barresi V and colleagues, where they genetically engineered neural progenitor clone ST14A to express cytosine deaminase (CD), by which 5-fluorocytosine (5-FC) can be converted into 5-fluorouracil (5-FU) to suppress tumor growth. DiI prelabeled CD-expressing ST14A cells were cotransplanted into rat brains with C6 glioma. The data showed that ST14A cells survived inside C6 tumor mass for at least 10 days and significantly reduced the size of tumor comparing to controls presumably through the action of 5-FU (Barresi et al., 2003).

## 5. Conclusions and perspectives

NSCs hold enormous promises for treating neurological diseases. However, at the present time, we are still facing many hurdles, one of which is how to direct these multipotent cells to become the desired cell types for a certain disease. NSPC clones proliferate rapidly in culture and maintain certain important characteristic properties after passages, therefore provide invaluable models for in vitro studies and transplantable tools for testing hypothesis in treating neurological disorders. Immortalized neural progenitor clones are potentially tumorigenic since they are engineered to express oncogenes such as Myc. Even though many reports have demonstrated that Myc expression is growth factor-dependent and can be down-regulated to an undetectable level after FGF2 withdrawal both in vitro and after transplantation, cautions have been taken to ensure the shutdown of oncogene expression upon cell differentiation. For example, modified immortalizing oncogenes such as the temperature-sensitive mutant of large T antigen, tsA58, have been used to generate a series of neural progenitor clones from the developing CNS. The tsA58 gene product is stable at permissive temperature (33$^{o}$C), but rapidly degraded when temperature goes higher such as body temperature in culture as well as after transplantation into animals. Generation of neural progenitor clones using controllable-Myc expression may provide another solution to the same problem. Human NSPC clones (summarized in Table 1) have been generated by tetracycline-controllable v-Myc (Kim et al., 2011; Sah et al., 1997) and c-MycER$^{TAM}$ transgene (Pollock et al., 2006). On the other hand, functions of promoting proliferation and tumorigenesis can be uncoupled in an experimental setting (Johnson et al., 2008). Therefore, dissecting out functional domains of oncogenes that enable sufficient expansion of neural stem cells and limit (or eliminate) their tumorigenic activity will be advantageous to generate newer version of immortalizing reagents (Harvey et al., 2007; Truckenmiller et al., 2002). A recent report on induced pluripotent stem cells (iPSCs) indicated that L-Myc, a Myc gene isoform, promoted iPSC generation with little transformation activity (Nakagawa et al., 2010). These safety-oriented designs in generation of immortalized progenitors will prove to be crucial in minimizing tumorigenc potential of exogenous oncogenes, and more research should be performed to optimize NSC immortalizing techniques especially in regard to safety for future clinical applications.

Oncogenes that are frequently used for NSC "immortalizations" such as Myc, carry mutations and are prone to spontaneous mutation as well. Some of these mutations potentiate their ability of transformation and tumorigenesis. Therefore, the culturing conditions for in vitro expansion of immortalized NSC clones need to be optimized and controlled in the goal of less stress so that spontaneous mutations can be minimized. The low oxygen culture condition, which many researchers have applied in their human stem cell culture, would fit in this type of precaution in addition to the fact that NSCs grow and differentiate better under this condition. Furthermore, a "quality check" protocol needs to be developed to screen out and eliminate cells with such mutations and tumor-like growth patterns in the case where mutations do occur. Stem cell replacement therapy is at its "transforming" stage. We are very hopeful for its future, but a lot more work needs to be done before it can go on to clinic, especially in regard to safety. Nevertheless, immortalized NSPC clones will for certain be the milestones in the road towards the ultimate goal of stem cell replacement therapy and, in some instances, may very well become therapeutics themselves (Thomas et al., 2009).

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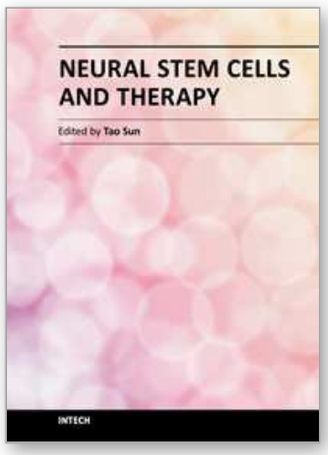


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This book is a collective work of international experts in the neural stem cell field. The book incorporates the characterization of embryonic and adult neural stem cells in both invertebrates and vertebrates. It highlights the history and the most advanced discoveries in neural stem cells, and summarizes the mechanisms of neural stem cell development. In particular, this book provides strategies and discusses the challenges of utilizing neural stem cells for therapy of neurological disorders and brain and spinal cord injuries. It is suitable for general readers, students, doctors and researchers who are interested in understanding the principles of and new discoveries in neural stem cells and therapy.



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