Published in final edited form as:

Birth Defects Res C Embryo Today. 2009 June ; 87(2): 182–191. doi:10.1002/bdrc.20149.

Gene regulatory networks in embryonic stem cells and brain development

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Abstract

Embryonic stem cells (ESCs) are endowed with the ability to generate multiple cell lineages and carries great therapeutic potentials in regenerative medicines. Future application of ESCs in human health and diseases will embark on the delineation of molecular mechanisms that define the biology of ESCs. Here we discuss how the finite ESC components mediate the intriguing task of brain development and exhibits biomedical potentials to cure diverse neurological disorders.

Keywords

embryonic stem cells (ESCs); gene regulatory network (GRN); neural stem cells (NSCs); glioma; induced pluripotent stem cells (ips)

Introduction

Embryonic stem cells (ESCs) cells are derived from the inner cell mass (ICM) of blastocytes during the early stage of embryonic development and offer tremendous opportunities in various aspects of human health (Keller, 1995; Thomson *et al.*, 1998). Two cardinal features of these cells *viz.* the ability to differentiate into diverse cell types of human body—a property called pluripotency and the self renewal capabilities have created enormous hopes in the area of regenerative medicines (De Coppi *et al.*, 2007; Hipp and Atala, 2008; Minino *et al.*, 2007; Srivastava *et al.*, 2008). For example in several neurodegenerative diseases, replacement of diseased brain cells with their normal counterparts holds promises for improving patients' health (Bachoud-Levi *et al.*, 2000; Kordower *et al.*, 1995; Sonntag and Sanchez-Pernaute, 2006). However, in such cases, neuronal transplantation has been limited by the use of fetal tissues (Mendez *et al.*, 2005; Sonntag and Sanchez-Pernaute, 2006). Besides, the ethical concerns pertaining to obtain embryonic brain tissues and the biological challenges associated with the proper functioning of implanted tissues offer grim prospects in transplantation medicines (Hipp and Atala, 2008; Sonntag and Sanchez-Pernaute, 2006). On the contrary, ESCs with unlimited potential of self renewal and ability to differentiate with neuronal phenotypes may provide continuous supply of donor cells for such studies (Brustle *et al.*, 1999; Riess *et al.*, 2007; Sonntag and Sanchez-Pernaute, 2006). The success of this endeavor will, however, rely much on the abilities of research strategies that are directed towards the delineation of cellular components and their interaction networks behind the functioning of ESCs. In personalized medicine such information will be of immense importance as well, as the modification and selection of ESCs with desired phenotypes could be performed based on specific demand. With the revelation of human genome and the advent of new technologies in the analysis of transcriptome and proteome

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powered by advance computational tools have provided research communities with the right impetus to gain deeper understanding in the pluripotency of ESCs.

Brain development- an example of ES cells pluripotency

Neurulation is the first event in organogenesis and begins from ESCs during the early events in embryonic development (Colas and Schoenwolf, 2001; Hornstein and Benvenisty, 2004; Schoenwolf and Smith, 2000). Most of our knowledge regarding the embryonic developments in mammals has come from the investigations of mouse ESCs (Martin, 1981). Under laboratory conditions ESCs aggregate to form embryoid body (EB) which undergoes further differentiation and forms three embryonic germ layers *viz.* ectoderm, mesoderm, and endoderm (Evans and Kaufman, 1981; Kurosawa, 2007; Reubinoff *et al.*, 2000). However, in contrast to a developing embryo, anterior-posterior or dorso-ventral pattern of differentiation has been found to be absent in EB despite the fact that EB cells respond to the ventralizing agents (Conley *et al.*, 2005; Lang *et al.*, 2004; Lyons *et al.*, 1991). In *in vivo* environment, ectodermal germ cells are further differentiated to form surface ectoderm and neuroectodermal regions (Rathjen *et al.*, 2001). The latter one subsequently forms the neural tube. Cells of neural tube are multipotent and undergo another level of differentiation to form lineage distinct cell types such as neurons, glial cells, and the cells of central nervous system (CNS) (Lang *et al.*, 2004; Rathjen and Rathjen, 2001). In conjunction with the surface ectoderm, neural tube cells form neural crest (NC). A salient feature of NC is its ability to respond and migrate towards the extracellular protein gradient formed by bone morphogenetic protein (BMP) (Liem *et al.*, 1995). It has been believed that the migratory abilities of NC in response to the signals emanating from neighboring tissues are responsible for the formation of peripheral nervous system (PNS) (Liem *et al.*, 1995). Recently it has been reported that adult brain also contains neural stem cells (NSCs) in the sub ventricular region (Reynolds and Weiss, 1992). Activities and fate of these cells are determined by the brain micro-environment partly controlled by BMP (Weiss *et al.*, 1996). Recently using the antagonists of BMP, it was observed that NSCs can be activated to form neurons in non-neurogenic region (Lim *et al.*, 2000).

Similar to a developing embryo, *in vitro* neuronal induction of EB has also been found to be mediated by bone morphogenetic protein (BMP) (Galli *et al.*, 2003). Recent reports indicate that the TGF (β) antagonists such as noggin, chordin, and follistatin may also activate the neuronal induction process (Gratsch and O'Shea, 2002). *In vitro* expansion of neural precursors has also been reported to be induced by stromal-cell-derived inducing activity (SDIA) and noggin (Kawasaki *et al.*, 2002). Carpenter *et al* has reported the use of retinoic acid and a combination of growth factors such as hEGF, hFGF2, hPDGF-AA, and hIGF1 for the enrichment of neural progenitors from human ES cells (Carpenter *et al.*, 2001). Majority of the cells in this study was found to be positive for N-CAM- a characteristic feature of neuron and A2B5 for neurons and glial phenotypes. In an analogous study, Brustle *et al* has reported the differentiation of neural cells to oligodendrocytes and astrocytes following the withdrawal of growth factors from the medium (Brustle *et al.*, 1999). Specific enrichment of serotonergic neurons from ESC derived progenitors has also been reported following the exposure of ESCs to ventralizing protein sonic hedgehog (SHH) (Ye *et al.*, 1998). Although detailed information related to the lineage differentiation in EB is lacking, similar embryonic developmental phases in EB has been postulated that progress through the rapid loss of pluripotency markers and developmentally restricted phenotypes (Palmqvist *et al.*, 2005; Singh *et al.*, 2008).

Recent studies indicate that cellular differentiation and morphogenetic events during embryogenesis coordinate with the differential expression of several genes on a temporal scale (Haub and Goldfarb, 1991; Pelton *et al.*, 2002; Rodda *et al.*, 2002; Rogers *et al.*, 1991;

Rosner *et al.*, 1990; Wang *et al.*, 2006). Based on such observation, efficacy of neural differentiation has been investigated by the use of lineage specific markers. For example, up regulation of *Oct4* is indicative of the pluripotency in ICM and early ectoderm formation whereas, down regulation of *Oct4* has been found to be associated with the loss of pluripotency, maturation of ectoderm, formation of progenitor of surface ectoderm, and neur ectoderm (Rathjen and Rathjen, 2001). Another set of genes *Sox1* and *Gbx2* have been found to be unregulated during the formation of neural tube while the down regulation of *Gbx2* has been found to be associated with the closure of neural tube (Lake *et al.*, 2000; Rathjen *et al.*, 2002). In undifferentiated neur ectoderm and neural progenitors, additional genes *viz.* *Sox1*, *Sox2*, *N-CAM*, *nestin* and *musashi1* have been observed to be over expressed (Aubert *et al.*, 2003; Rathjen and Rathjen, 2001). During anterior-posterior specification, *Hoxc5* and *Hoxc6* demarcate the areas of neural tube that are committed to form spinal cord (Wichterle *et al.*, 2002). Similarly *Nkx6.1*, *Olig2*, *Dbx1*, *Irx3*, and *Pax6* were found to be associated with the dorso-ventral specification (Wichterle *et al.*, 2002). Enrichment of radial glial cells was found to be associated with the *Pax 6* and *Shh* over expressions and *Pax 7* down regulation (Ogawa *et al.*, 2005; Wichterle *et al.*, 2002). Neural induction was, however, found to be antagonized by the over expression of *Wnt* and other members of *Wnt* signaling pathways such as *Sfrp2* (Aubert *et al.*, 2002). Differentiation of neurons such as motor neurons and dopaminergic neurons are also incumbent upon the regional activities of gene expression. For example, *Islet1* expressing progenitor cells of neural tube form motor neurons (Pfaff *et al.*, 1996) while *Shh* and *FGF8* signaling (Ye *et al.*, 1998) regionalize ventral midbrain for the formation of dopaminergic neurons (Mizuseki *et al.*, 2003). Enrichment of the latter form has also been reported from the activities of nuclear receptor 1 (*Nurr 1*) from ventrally committed mesencephalic progenitors as exemplified by the dopaminergic neuron markers *TH* and *AADC* (Simon *et al.*, 2003). In this regard specific response of *Nurr 1* to dopaminergic neuron could be mentioned as no γ -aminobutyric acid (GABA), ChAT, serotonergic, and glutamate⁺ neurons were observed following the overexpression of *Nurr 1* (Kim *et al.*, 2002).

Key transcription factors or signaling events

Several recent studies have highlighted that biological regulation of brain development is organized in a hierarchical and combinatorial fashion which involves the interplay between genes and proteins (Dreyer, 1998; He and Rosenfeld, 1991; Le Novère, 2007; Lumsden, 1995; Ooi and Wood, 2008; Sandell and Trainor, 2006). Central to this finding is the occurrence of local non-heritable epigenetic changes that impart additional level of control for proper brain development (Mehler, 2008). However, greater appreciation of such complex network lies in understanding how finite network components can drive the stunning complexities in brain. Therefore, current research efforts are directed towards determining changes in the network modules on a spatiotemporal scale and the assignment of functional annotation to such changes (Hood, 2008; Hwang *et al.*, 2009; Villoslada *et al.*, 2009). In future, such study will provide us cues about the structural and cognitive development of brain.

The series of events during the formation of NC and its differentiation could be cited as an example to emphasize the role of GRN in brain development (Mayor *et al.*, 1995). In general, NC specification could be divided into four distinct phases *viz.* induction, maintenance of multi-potency by cell-cycle control, epithelial-mesenchymal transition, and differentiation (Meulemans and Bronner-Fraser, 2004; Sauka-Spengler and Bronner-Fraser, 2008). Each step of NC specification is under the tight control of transcription factors that not only orchestrate the expression of network components within the GRN but also cross-talk with each module. NC induction begins in response to *wnt* signal which originates from the underlying mesoderm and FGF (fibroblast growth factor) gradient that appears from the

neighboring mesoderm and ectoderm (Garcia-Castro *et al.*, 2002; Monsoro-Burq *et al.*, 2003). A panel of genes that marks the induction of neural border plate- an intermediary step in the differentiation events includes *Msx1*, *Msx2*, *Pax3*, *Pax7*, and *Zic1* (Monsoro-Burq *et al.*, 2005; Sato *et al.*, 2005). Induction events follow NC specification, which is marked by the expression of genes such as *Snail*, *FoxD3* and members of *SoxE* (Taneyhill *et al.*, 2007; Taylor and Labonne, 2005). Among the members of *SoxE*, *Sox9* promotes the survival of multi-potent NC progenitors by up-regulating anti-apoptotic gene *Snail* (Sakai *et al.*, 2006). Multi-potency of NC progenitors is maintained by c-*Myc-Id* axis (Kee and Bronner-Fraser, 2005), and together with other NC differentiation markers such as *Sox10*, *FoxD3* and *AP2*, NC subpopulation begins epithelial to mesenchymal transition (Thiery and Sleeman, 2006). Adhesive properties and migratory properties of NC population is tightly controlled by a number of downstream membrane proteins such as ADAM 10, integrins, neuropilins, Eph and some matrix metalloproteases (Alfandari *et al.*, 2001; Lallier and Bronner-Fraser, 1993; Shoval *et al.*, 2007; Smith *et al.*, 1997).

GRN in the self-renewal of NSC is another area of active research in the study of brain development. NSCs retain the ability to self-renew and proliferate, and can generate both neuronal and glial lineages (Gage *et al.*, 1998; McKay, 1997; Shi *et al.*, 2008). Among the different transcriptional regulators of NSCs, *NR2E1* (*TLX*)-an orphan nuclear receptor has been drawing considerable interests due to its ability to empower NSC with the self-renewal capability in the adult brain (Shi *et al.*, 2004). *TLX* knock-out mice exhibited a number of brain anomalies including defects in neurogenesis, reduced cerebral hemispheres, and severe retinopathies (Monaghan *et al.*, 1997; Uemura *et al.*, 2006; Yu *et al.*, 2000). At cellular level *TLX*-positive cells isolated from adult brain were found to have proliferative and self-renewing abilities whereas, *TLX*-null cells from mutant mice lacked such abilities. In addition, when *TLX*-null cells were re-programmed with *TLX*, stemness was rescued indicating the unique developmental role that *TLX* might play (Shi *et al.*, 2004). Recently Sun *et al* proposed a possible molecular mechanism behind *TLX* mediated self-renewal. *TLX* was found to repress transcriptional expression of cyclin-dependent kinase inhibitor, *p21* and tumor suppressor *pten* thorough the recruitment of histone deacetylases (HDAC)(Sun *et al.*, 2007). Other *TLX* downstream effectors which may impart additional control on the NSC proliferation may include *GFAP*, phospholipase C, MAPK, and *Atm1* (Shi *et al.*, 2004; Zhang *et al.*, 2006).

Besides *TLX*, other nuclear receptors such as estrogen receptor (*ER*), peroxisome proliferator-activated receptor γ (*PPAR* γ), *N-COR*, and thyroid hormone receptors (TR) have also been known to play prominent roles in the NSC self-renewal, proliferation, and differentiation (Ambrogini *et al.*, 2005; Fowler *et al.*, 2005; Katayama *et al.*, 2005; Kishi *et al.*, 2005). For example premature differentiation of NSCs to astrocytes and reduced self-renewal was observed in *N-COR* gene disrupted mice (Hermanson *et al.*, 2002). *In-vitro* induction of NSCs with cytokine revealed activation of *PI3K-Akt* dependent phosphorylation of *N-COR* which may throw some lights about the possible molecular mechanism that keeps NSCs in an undifferentiated state (Hermanson *et al.*, 2002). Similar developmental role is also played by the *Sox* family of DNA binding proteins. In proliferating NSCs, *Sox* family of proteins such as *Sox1*, *Sox2* and *Sox3* are ubiquitously expressed during all developmental phases starting from infant to adult brain (Uchikawa *et al.*, 2003; Zappone *et al.*, 2000). *Sox2/Sox3* in particular drives undifferentiated state of NSCs as observed following over expression of these proteins in neural progenitors that arrested further differentiation of these cells (Pevny and Placzek, 2005). On the contrary *Sox2/Sox3* dominant negative induced neuronal differentiation (Graham *et al.*, 2003). Using regulatory mutations of *Sox2* genes Ferri *et al* showed that heterozygotes developed certain features of neurodegeneration which are similar to many human neurodegenerative diseases. Impaired neurogenesis of adult brain was also observed in the same study (Ferri *et al.*,

2004). *Hes1* and *Hes5* are other effectors of NSC stemness (Ishibashi *et al.*, 1994; Ohtsuka *et al.*, 2001). The latter genes repress the expression of activator/differentiation genes such as *Mash1*, *Math* and *Neurogenin* (Kageyama *et al.*, 2005) and thus actively participate in the maintenance of NSCs self-renewal. *Bmi1* is another transcriptional repressor that maintains adult stem cell features by acting as an inhibitor of *p16^{Ink4a}* and *p19^{Arf}* (Molofsky *et al.*, 2005).

Chromatin remodeling and epigenetic control also play important role in the self-renewal and differentiation of NSCs. Use of HDAC inhibitors which prevent the binding of transcription factors to the DNA, promotes neuronal differentiation (Hsieh *et al.*, 2004). Transcriptional regulation of many neuronal genes through REST (RE1 silencing transcription factor) which binds to conserved RE1 binding site is mediated through the recruitment of HDAC complexes (Ballas *et al.*, 2005; Lunyak and Rosenfeld, 2005). In a recent study Singh *et al* has shown that higher level of REST expression has been correlated with the self-renewal capabilities of ESCs through the targeting of miRNAs miR-21 (Singh *et al.*, 2008). The study indicated that miRNAs might represent another tier of control in the self-renewal and differentiation of ESCs and highlighted the need for applying system approaches that would propel the complete elucidation of underlying biological network maintained at the levels of DNAs, RNAs and proteins.

Transcription factors in ES pluripotency

While above studies were directed towards understanding the multipotency of NCs and NSCs, maintenance of pluripotency in ES cell could also be mentioned to highlight the crosstalk between different transcription factors and their targets. Recently Kim *et al* described an *in vivo* biotin-tagging approach that can identify targets of multiple transcription factors associated with the pluripotency in ESCs (Kim *et al.*, 2008a). Using mouse ESC cell lines expressing nine *in vivo* biotinylated transcription factors *viz.* *Nanog*, *Sox2*, *Dax1*, *Nac1*, *Oct4*, *Klf4*, *Zfp281*, *Rex1*, and *Myc* the same group identified over 400 promoter regions occupied by more than four transcription factors. Among the nine transcription factors, *Rex1* and *Myc* targets fell in different clusters than that of the rest pluripotency factors. The study provided a working model to predict the status of ES cells differentiation based on the transcriptional control of targets if occupied by four or more pluripotency factors (Kim *et al.*, 2008a).

Wang *et al* described a protein interaction network relevant to maintaining pluripotency in mouse ESCs (Wang *et al.*, 2006). Using *Nanog* c-DNA and *in vivo* tandem tagging approach that employed N-terminal Flag and biotin linkers, 30 interacting proteins were identified by tandem mass spectrometry. In the same study, a group of seven transcription factors *viz.* *Sall1*, *Sall4*, *Rif1*, *Tif1B*, *Mybbp*, *Dax1* and *Nac1* which were appeared in tandem purification and single linker purification was designated as the major *Nanog*-interacting partners. *Oct4* was found to be one among other transiently interacting *Nanog*-partners. Two previously undescribed *Nanog*-interacting factors *viz.* *Nac1* and *Zfp281* and their target genes relevant to ES cells functionality were also identified in this study.

In figure 1 we have presented a tentative network of core pluripotency factors and their targets. Notably the hubs with more than two occupancies such as *Nanog*, *Sox2*, *Oct4*, *Mybl2*, *Rarg*, and *Cbx1* may deserve more attentions in the context of ES cells pluripotency.

The ability of certain transcription factors to induce pluripotency is recently highlighted independently in the work of Takahashi *et al* (Takahashi *et al.*, 2007) and Park *et al* (Park *et al.*, 2008). Using four transcriptional factors *viz.* *Oct4* (*Pou5f1*), *Sox2*, *Klf4*, and *Myc* both groups were able to induce pluripotency in mouse somatic cells. More interestingly, the same set of transcription factors was used to reprogram human skin cells to induced-

pluripotent cells (iPS) indicating the existence of common network in pluripotent mouse and human cells (Park *et al.*, 2008; Takahashi *et al.*, 2007). A two-factor combination such as *Oct4* and *Klf4/Myc* was also shown to induce pluripotency in mouse NSCs (Kim *et al.*, 2008b). Very recently, the same group has reported pluripotency induction in murine NSCs by using *Oct4* alone (Kim *et al.*, 2009). The induced cells (1F iPS) resembled the features of embryonic stem cells and formed committed progenies such as cardiomyocytes, NSCs, and germ cells when allowed to differentiate *in vitro*. In *in vivo* condition these cells formed teratoma and caused germline transmission similar to that of embryonic stem cells as well. By avoiding oncogenic proteins such as *Myc* and *Klf4* which otherwise might develop tumor, the above study increased the clinical potential of the approach. Altogether, application of pluripotent factors to induce stemness in somatic cells has opened up new avenues in regenerative medicines to generate pluripotent stem cells from host specific somatic cells. However, extensive knowledge on the composition and dynamics of pluripotency factors and molecular mechanisms behind their ability to interact with DNA and proteins to control cellular differentiation will provide more access to the utility of pluripotent stem cells in medicines.

State of disordered NSC- glioma as an example

Gliomas including glioblastoma (GBM) and anaplastic astrocytoma are notorious tumors in the CNS and the median survival rate even under treatments could only be extended to only 12–15 months (Dent *et al.*, 2008; Robins *et al.*, 2007). Gliomas can be classified into two major sub groups *viz.* primary glioma that appears *de novo* and secondary glioma that arises from a slow progressing low grade glioma (Tso *et al.*, 2006). Genetically these two types of gliomas are distinct and each exhibit characteristic genomic mutations. For example 60% of secondary gliomas harbor mutations in *PDGF* and *p53*, while primary gliomas are characterized by the mutations in *EGFR*, *PTEN* and *INK4A/ARF* (Zheng *et al.*, 2008). In contrast to popular belief that gliomas arise from glial cells in the brain parenchyma, a growing body of evidence indicate that a sub-population of cells within the tumor are empowered with the capacity to form cancers in immunodeficient mice (O'Brien *et al.*, 2007; Singh *et al.*, 2003). In the current paradigm, NSCs with the characteristic expression of *CD133*⁺ have been considered to be responsible for gliomas (Singh *et al.*, 2004). Experiments with *CD133*⁺ NSCs isolated from gliomas indicated that these cells can form floating aggregates called neurospheres and retain self-renewal ability (Gage, 2000). Clinically the increased expression of *CD133*⁺ has been found to be associated with higher grade of gliomas (Perez Castillo *et al.*, 2008). Along with the *CD133*⁺ expression, these cells also express other NSC markers such as *nestin* and *notch* (Singh *et al.*, 2004). In agreement with NSCs capabilities, *CD133*⁺ cells are multipotent and can be differentiated to form neurons, astrocytes and oligodendrocytes (Gage, 2000). Recently Bao *et al.* has reported that increased radio-resistance of *CD133*⁺ cells is associated with higher DNA repair capacity and preferred cell cycle checkpoint control (Bao *et al.*, 2006). This is an unmatched feature of normal stem cells where the growth of the cells and self-renewal capability is tightly controlled. Therefore, it appears that cancer stem cells in gliomas might be originated from disordered or transformed NSCs. In this regard it is noteworthy that *Nf1* and *Trp53* deletions in mouse neural stem cell formed gliomas (Zhu *et al.*, 2005). Similarly *p53* and *PTEN* deletions in mouse NSCs promote undifferentiated phenotypes, high renewal capabilities and increase *Myc* expression-characteristic features of gliomas (Zheng *et al.*, 2008). However, the molecular mechanisms behind such neoplastic transformation are not clearly understood and it represents an ongoing research debate. In parallel it has also been proposed that other committed progenitor and even terminally differentiated cells may also remain involved in gliomas (Vescovi *et al.*, 2006). Ability of NSCs to give rise to unanticipated cell types such as muscle, endothelial and hematopoietic cells (Bjornson *et al.*, 1999; Galli *et al.*, 2000; Wurmser *et al.*, 2004), and mesenchymal stem cells to astrocytes

and neurons (Kopen *et al.*, 1999; Woodbury *et al.*, 2000) indicated the existence of overlapping genetic circuits in different stem cells and much broader differentiation inventory. At the same time increased understanding of how stem cells communicate with their niche and how niche components shape the proper development of progenitor cells would provide additional clues about the transformation, growth and maintenance of cancer stem cells.

Potential of stem cell-therapy in various brain diseases

From previous discussion it seems apparent that GRN together with intrinsic and extrinsic signals orchestrate proper brain development. The flip side of such observation is that diseases may appear from perturbed GRN and/or altered gene and protein expression profiles. In neuronal diseases which are otherwise apathetic to conventional treatment, the key medical aim will be to develop efficient replacement strategies that would restore normal brain functions. While paucity of organ donors in replacement therapy has been appeared to be a major challenge in regenerative medicines, the ability of ES cells in replenishing a particular cell population that is defective due to disease, injury or anomalies in development has propelled new hopes. In the context of neurodegenerative diseases where one or several neuronal populations become progressively deteriorated or dysfunctional, stem cell therapy seems highly appealing (Hipp and Atala, 2008; Sonntag and Sanchez-Pernaute, 2006; Srivastava *et al.*, 2008). Parkinson's disease (PD) for example, is characterized by the loss of dopaminergic neurons (DA) in the substantia nigra pars compacta (SNpc) and the neuronal dysfunction is observed among the surviving neurons due to the formation of lipid and protein aggregates called Lewy bodies (Braak *et al.*, 2003). Neuronal transplantation in PD has recently been possible by the use of fetal mesencephalic tissue (Kordower *et al.*, 1995; Mendez *et al.*, 2005). An alternate and viable approach will be to use ESCs and differentiate them into DA (Amit *et al.*, 2000). In mouse model of PD, Kim *et al* showed that ES-derived midbrain NSCs when transplanted, exhibited similar electrophysiological and behavioral properties of midbrain neurons (Kim *et al.*, 2002). In a similar approach Bjorklund *et al* examined the potential of brain microenvironment or niche in the selection of committed progeny cells (Bjorklund *et al.*, 2002). In their study Isacson's group used undifferentiated mouse ES cells and following transplantation to rat striatum, ESCs were spontaneously differentiated to form DA cells which rescued cerebral function in mouse model of PD (Bjorklund *et al.*, 2002). Their results clearly indicated that the niche environment in the brain might promote the development of region specific neuronal cells.

The ability of transplanted fetal striatal neurons to synthesize GABA in animal model of Huntington's disease (HD) has also illuminated the idea of using ESC-derived population in place of fetal tissues for continuing supply of striatal projection neurons (Bachoud-Levi *et al.*, 2000; Freeman *et al.*, 2000). Similar exogenous supply of GABA to brain was also examined recently (Gernert *et al.*, 2002). By mimicking epileptic conditions in rat, Gernert *et al* has shown that mouse cortical neurons engineered to produce GABA when transplanted in rat central piriform cortex, exhibited long term anticonvulsant effects (Gernert *et al.*, 2002). Therefore, in epilepsy, ESC-derived cells may also offer a potential therapeutic solution to restore normal excitability in brain (Muotri, 2009). In traumatic brain injury, ESCs can equally provide a renewable source of cells to suffice depleted or damaged neuronal populations. As exemplified in several recent publications, transplanted ESCs might also improve motor functions in animal models of brain injuries (Longhi *et al.*, 2004; Riess *et al.*, 2007).

Re-myelination of demyelinated brains tissues as observed in several degenerative diseases such as multiple sclerosis and vascular leukoencephalopathy or in spinal cord injury is also an active area of research where ESCs can be proved useful (Nistor *et al.*, 2005). Both ESC-

derived oligodendrocyte progenitor cells and undifferentiated ESCs were used to show the potential of re-myelination *in vivo* mouse models (Brustle *et al.*, 1999). However, the key questions that still remain unanswered are the stability of such transplanted cells and the possibility of de-differentiation. Additionally, obtaining a clean and homogeneous population of differentiated cells from ESCs is still an uphill task (Sonntag and Sanchez-Pernaute, 2006). However, the paradigm of cell selection could be matured by identifying novel cell surface markers and connecting the surface expression to the functional activities. In this regard new advancement in gene manipulating technologies such as RNAi, site specific recombinases and tetraploid aggregation (Kunath *et al.*, 2003) may help in manipulating and examining desired cellular phenotypes from ESC-derived progenies. The information obtained from such studies could be extended to the analysis of neurodegenerative diseases in a multi-parameter *in vivo* environment.

Besides implications in transplantation therapy, ESCs can also be used as delivery vehicles to supply neurotrophic factors in brain with the idea of modifying the progression of neurodegenerative diseases. Neurotrophic factors being proteins pose a delivery problem because of the blood-brain-barrier (Sonntag and Sanchez-Pernaute, 2006). Arenas group recently used engineered NSC expressing persephin (PSP)-a glial-derived neurotrophic factor (GDNF) that promotes the survival of neuronal cells, to rescue the cerebral functions of DA neurons in PD (Akerud *et al.*, 2002). In their study NSC was not only integrated in the neuronal circuits of brain but also released PSP and differentiated into astrocytes, oligodendrocytes, and neurons indicating, thereby, the unlimited potential of stem cells in treating various forms of neurodegenerative diseases (Akerud *et al.*, 2002). Another potential area of delivering neurotrophic factors could be gliomas. Recently Piccirillo *et al* has shown that BMP can promote the differentiation of CD133+ cells, thereby seriously weakening their cancer causing abilities (Piccirillo *et al.*, 2006). Thus establishing a rout to provide constant supply of biological agents that would slow down the cancer progression will be of very high demand.

As an approach to treat Alzheimer's disease (AD) in human subjects, autologous fibroblasts were engineered *ex vivo* to produce nerve growth factor (NGF) and transplanted into the cortex of patients suffering from Alzheimer's disease (AD) (Tuszynski *et al.*, 2005). Such transplantation induced the ramifications of cholinergic neurons in the transplanted area and improved cognitive development of AD patients (Tuszynski *et al.*, 2005). Although application of ESCs to such extent is lacking, it carries tremendous potential of delivering biological agents such as genes, anti-apoptotic factors, anti-oxidants, growth factors or even antibodies to CNS (Bowers *et al.*, 1997; Sonntag and Sanchez-Pernaute, 2006; Srivastava *et al.*, 2008).

Future perspectives

Systems Biology with its empowering technological platforms have allowed global measurement of biological information at the levels of DNA sequence (high throughput next generation sequencing), transcript level (DNA arrays), protein abundance (mass spectrometry MS and protein arrays), protein-DNA (ChIP-Chip/ChIP-Seq) and protein-protein (affinity purification and MS) interactions. Systematic integration of all these information into gene regulatory networks has enabled better understanding of ES cell pluripotency, and more recently provided foundational new insight for the pathophysiology and potential new diagnosis in a degenerative brain disease (prion) mouse model (Hwang *et al.*, 2009). We envision that the systems approach to biology, and eventually systems approach to disease, will see increasing applications in numerous other diseases, in particular neurological disorders given their intrinsic complexity, leading to unprecedented deep understanding and prevention of the disease.

Acknowledgments

Gene regulatory network of core ES cell pluripotency factors. These core factors include Nanog, Sox2, Dax1, Nac1, Oct4, Klf4, and Zfp281. They act in a highly combinatorial fashion and activate or maintain expression of their target genes. Targets represented in pink can be bound by all 7 core ES cell pluripotency factors.

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Figure 1.