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Recent advances in neural stem cell in livestock

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Abstract
An organ or tissue protrudes through an opening in the body wall, causing a hernia. A tear in the abdomen wall or diaphragm may cause the aperture, or it may be a natural opening such as the inguinal canal or femoral canal. The protruded tissue is covered by the skin, unlike in a prolapse. A typical hernia has a hernial ring and a hernial sac through which the contents have migrated. The neck, body, and fundus make up the hernial sac, which encloses the hernial contents. A 3-month-old cross-bred buffalo bull calf was brought with a history of swelling in the umbilical region. Upon physical examination, the condition was diagnosed as umbilical hernia. The umbilical hernia was reduced and corrected by performing herniorrhaphy. The animal had an uneventful recovery.

Keywords: Hernia, buffalo bull, umbilical, herniorrhaphy

Introduction
Stem cells (SC) are defined by two characteristics: they are multi potent and have the property of self-renewal. Embryonic stem cells are cells arising from the dividing zygote at the start of ontogenetic development which are capable of forming cells of various tissues by cell division until final differentiation. The nervous system is a complex organ made up of neurons and glial cells, which surround and support neurons. Neurons send signals that affect numerous functions including thought processes and movement. All cells present in the central nervous system (CNS) are originally derived from the early neuroepithelium that forms as the neural plate along the midline of the developing embryo. The differentiation of the neuroepithelial stem cells into neurons and glia then proceeds in a temporal specific manner that is specific for each region of the developing neural tube (McConnell, 1995; Rao, 1999) [29, 39]. It has been established that active neurogenesis, a process of generating functionally integrated neurons from undifferentiated, multipotent progenitor or stem cells, continues in discrete regions of the adult CNS throughout the life of all mammals, including humans (Gage, 2000; Temple, 2001) [15, 45]. The adult vertebrate central nervous system (CNS) consists of four major differentiated cell types: neurons, astrocytes, oligodendrocytes and ependymal cells. As development proceeds and compartmentalization of the CNS becomes apparent, neural stem and early progenitor cells in the mammalian fetal CNS are considered to be concentrated in seven major areas: the olfactory bulb, ventricular zone (VZ) and subventricular zone (SVZ) of the forebrain; the hippocampus, cerebellum, cerebral cortex and the spinal cord. Their number and pattern of development vary in different species (Gage, 2000) [15]. In recent years, it has become clear that the adult mammalian brain retains the capacity to generate new neurons and glia throughout the life of the organism (2004 Doetsch *et al*., 1999; Alvarez-Buylla and Lim,) [2]. NSCs – as rigorously defined – are multipotent cells with the ability to self-renew and to generate mature, differentiated daughter cells of all neural lineages throughout the developing neuraxis (including neurones of multiple subtypes, astrocytes and oligodendrocytes) as well as to reconstitute those cell types in ablated neural regions (Parker *et al*., 2005) [34]. NSCs is not only to participate in organogenesis but also to maintain homeostasis throughout life, the NSC is endowed with an intrinsic plasticity that allows it to shift its fate dynamically in response to cues from the local micro-environment *in vivo* (Reynold and Weiss,1992; Mckay,1997) [40, 30]. The progeny of NSCs can integrate functionally into the surrounding host neural structures for nervous system repair In the adult, NSCs are generated for the continuous replacement of specific classes of brain neurons (i.e. periglomerular and granule cells in the olfactory bulb and granule cells of the dentate gyrus) but also in response to neurodegenerative disease, traumatic injury, stroke, etc. (Schouten *et al*., 2004; Park *et al*., 2006).

Sources of Neural stem cell (NSC)

NSC have been assumed to exist only in the embryonic nervous system at the beginning of neural tube formation. NSC are present in the central nervous system (CNS), in the peripheral nervous system (PNS) and in neural crest cell populations. When the telencephalon of the mouse becomes vascularized around day 10 of gestation, estimates of NSC in this region range from 5 to 20% of total cell number (Kilpatrick *et al*., 1995; Temple, 2001) [45, 24]. The number of NSC declines during embryonic development. Germinal regions persist in the adult mammalian brain that is capable of generating new neurons: the subventricular zone (SVZ) of the lateral ventricle, and the subgranular zone (SGZ) of the hippocampal formation and, to some extent, the spinal cord. Progenitor cells in the mammalian fetal CNS are considered to be concentrated in seven major areas: olfactory bulb, ventricular zone (VZ) and subventricular zone (SVZ) of the forebrain, hippocampus, cerebellum, cerebral cortex and spinal cord. Progenitor cells number and pattern of development vary in different species (Gage, 2000) [15]. Motoneurons are among the first neurons to develop (Phelps *et al.*, 1996) [35]. The early studies led to the isolation of stem-like cells from the embryonic mammalian central nervous system (Temple, 1989) [44]. After the discovery of neural stem cells in the embryo, the first isolation of stem-like cells from adult brain (Reynolds and Weiss, 1992) [40] began new era of neuroscience. (Adult neural stem cells have now been found in the two principal adult neurogenic regions, the hippocampus and the subventricular zone (SVZ), and in some non-neurogenic regions, including spinal cord (Gage, 2000) [15]. PNS neural crest stem cells express the low-affinity neurotrophin receptor p75 (Stemple and Anderson, 1992) [42]. When the neural plate first emerges, does it consist solely of stem cells or does it includes both stem cells and restricted progenitor types? It is uncertain. In spinal neural tube from embryonic day 8 (E8) rat, over 50% of the viable cells at 24 hours are stem cells (Kalyani *et al*., 1997, 1998; Qian *et al*., 2000) [22, 38]. In telencephalon of E10 mouse, estimates of stem cells range from 5 to 20% (Kilpatrick and Bartlett, 1993) [24]. Early neuroepithelial cells are columnar, touching ventricle and pial surfaces during the cell cycle. Clonal studies suggest that most glia, both astrocytes and oligodendrocytes, originate from stem cells (Rao, 1999) [39]. Neurospheres generated from different CNS regions express region-specific markers, indicating that the original stem cells are regionally specified. Regulatory sequences control region-specific expression of the transcription factor Sox2, so that expression is seen in telencephalic but not spinal cord stem cells (Zappone, 2000) [54]. Different neural cell types arise in a precise temporal order that is characteristic for a particular region and species. CNS stem cells undergo repeated asymmetric cell divisions, first producing neurons then glia (Qian *et al*., 2000) [38]. Early neural crest stem cells generate significantly more neurons than later stage cells: like CNS stem cells, their neurogenic capacity declines with stage (White *et al*., 2001) [48]. Uchida *et al*. (2000) [47] isolated the neural stem cell from the human fetal spinal cord and brain tissue. Azari *et al*. (2010) [3] isolated and expanded NSCs from adult mouse periventricular region the harvested tissue is first chemically digested using trypsin-EDTA. Konagaya *et al*. (2011) [25] have designed the culture substrate for large scale expansion of neural stem cells. They synthesize epidermal growth factor fused with a hexahistidine sequence (EGF-His) and a polystyrene-binding peptide (EGF-PSt), and these engineered growth factors were surface-anchored to a nickel-chelated glass plate and a polystyrene dish, respectively. Neurosphere-forming cells prepared from the fetal rat striatum were used to examine the selective expansion of NSCs using the EGF-His-chelated module and the EGF-PSt-bound polystyrene dish (Konagaya *et al*., 2011) [25]. Neural progenitor cells isolated and characterized from postmortem human cortex (Schwartz *et al*., 2003) [41]. Neural precursors are relatively resistant to post-mortem ischemic and oxidative stress compared with neurons (Safar, 1979) [40].

Characterization of Neural Stem Cells

Cell specific markers are a valuable tool in tracing neural stem cells during development. Markers allow the identification of NSCs in cell culture. A novel approach to acquire new markers is via cDNA microarray analysis, which may yield new specific markers (Kornblum and Geschwind, 2001) [26].

Nestin

Nestin is the most frequently used and highly accepted marker for NSC. It is a class IV intermediate filament protein and was found to be expressed predominantly in stem cells of the CNS (Frederikson *et al*., 1988) [13], but not in mature CNS cells. Nestin expression, neither restricted to the embryonic CNS, nor the progenitor cells of neurons, but can be found in the PNS and in a number of other non-neural stem cell populations. Among these are pancreatic island precursor cells, myogenic cells, hair follicle sheath progenitor cells and embryonic stem cell-derived progenitor cells that have the potential to develop into neuroectodermal, mesodermal and endodermal cell lineages (Wiese *et al*., 2004; Li *et al*., 2003) [50]. Nestin seems to play a role in the structural organisation of cells where it probably participates in remodelling processes (Michalczyk and Ziman, 2005). Li *et al*. (2005) expressed nestin in cells isolated from human fetal striatum. Zhang *et al*. (2006) [55] expressed nestin in cells isolated from rat brain.

Sox2

Sox2 is a “founder member” of the Sox gene family. Sox2 can also re-establish pluripotency in terminally differentiated cells reprogramming them to induced pluripotent stem cells (iPS).Sox2 express in in the developing central nervous system (CNS) (Collignon *et al*., 1996) [9]. Sox2 expression is also detected in the postnatal neurogenic regions in the subventricular zone (SVZ) and hippocampus dentate gyrus (DG) (Ellis *et al*., 2004) [11]. Sox2 function to maintain neural precursor cell properties. Sox2 regulate the Notch pathway which is responsible for maintenance of neural stem cells (Bani-Yaghoub *et al*., 2006) Sox1 and Sox3, related genes whose expression overlaps with that of Sox2 (Pevny and Placzek, 2005), compensate for much of its function in the embryonic CNS. Sox2 ablation at developmental stages earlier than E12.5 will be required to determine if compensatory mechanisms are equally effective in early neural tube development. Sox2 is required for neuronal differentiation in eye and brain. Expression of Sox2 is generally localized to undifferentiated precursors, and, though it is retained in some populations of differentiating neurons, expression is generally down regulated with differentiation. Sox2 acts in neuronal progenitors to down regulate genes of an alternative (glial) differentiation fate (Cavallaro *et al*., 2008) [8]. Cancer stem cells, a tumor cell subpopulation able to

re-initiate a tumor, may depend on genes important for normal neural stem cells. Sox2 is expressed in brain tumors (Gangemi *et al*., 2009) [16], and its roles in normal neural stem cells suggest that cancer stem cells may maintain the requirement for Sox2 exhibited by normal neural stem cells (Favaro *et al*., 2009). Li *et al*. (2005) expressed Sox2 in cells isolated from human fetal striatum.

RNA-binding protein Musashi-1

Musashi is an evolutionarily conserved family of RNA-binding proteins that is preferentially expressed in the nervous system. (Okano *et al*., 2002) [33] The first member of the Musashi family was identified in Drosophila. This protein plays an essential role in regulating the asymmetric cell division of ectodermal precursor cells known as sensory organ precursor cells. Level of expression of the RNA-binding protein Musashi-1 is selectively higher in NSCs than in neural precursor cells (Kaneko *et al*., 2000) [23]. Musashi-1used as a marker and succeeded in identifying NSCs and progenitor cells in the adult human brain (Pincus *et al*., 1998) [37]. Musashi-1 protein induces transactivation of the promoter of the Notch signal target Hes-1 gene (Okano *et al*., 2002) [33]. Activation of the Notch signal positively regulates neural stem-cell self-renewal (Hitoshi *et al*., 2002) [21].

Pax 6

Members of Pax family proteins are HD (homeodomain)-containing transcription factors, and Pax6 is the most characterized member (Gehring and Ikeo, 1999) [17]. Transcription factor Pax6 plays an important role in fate determination of neural progenitor cells in animal models; yet, its distribution and role in the human developing brain have not been reported. During development, *Pax6* is expressed in the dorsal forebrain, including cortex, dorsal thalamus and pretectum, and functions in patterning the brain. Li *et al*. (2005) expressed Pax6 in cells isolated from human fetal striatum.

p75 Neurotrophin R (NTR)

p75 NTR, also named low affinity nerve growth factor (NGF) receptor, is a type I transmembrane protein that belongs to the tumor necrosis factor receptor superfamily. Neural crest stem cells (NCSCs) have been isolated based on their surface expression of p75NTR (Stemple *et al*., 1992) [42]. In addition, neuroepithelial-derived p75NTR$^+$ cells are also able to differentiate into neurons, smooth muscle and Schwann cells in culture (Mujtaba *et al*., 1998) [31]. p75 Neurotrophin expressed In cell isolated from rat brain (Young *et al*., 2007) [52].

PSA-NCAM (Polysialic acid-neural cell adhesion molecule)

The regulated expression of neural cell adhesion molecule (NCAM) isoforms in the brain is critical for many neural developmental processes. The embryonic form of NCAM, PSA-NCAM, is highly polysialylated and is mainly expressed in the developing nervous system. PSA-NCAM may be related to synaptic rearrangement and plasticity (Muller *et al*., 1996) [32]

CD 133

Widely used as a marker for identification and isolation of neural precursor cells from normal brain or tumor tissue (Sun *et al*., 2009) [69]. CD133 is CD133 is a 120 kDA five membrane domain glycoprotein and is expressed on immature hematopoietic stem and progenitor cells and on progenitor cells of the non-hematopoietic system, such as NSC (Uchida *et al*., 2000) [47]. CD133 expression could also be detected in brain tumours (Singh *et al*., 2003). Li *et al*. (2005) expressed CD133 in cells isolated from human fetal striatum.

Factor affecting growth and multi-fication of neural stem cells

Survival, proliferation, and differentiation of individual stem cell colonies can be monitored in the presence of different growth factors in culture, allowing us to distinguish between the instructive and selective effects of growth factors. This provides the opportunity to study how individual growth factors influence the process of lineage determination. In NCSCs, bone morphogenic proteins (BMPs) instruct neuronal differentiation, neuregulin (Nrg-1; also known as glial growth factor) instructs glial differentiation, and transforming growth factor instructs myofibroblast differentiation. In CNS stem cells, in which BMPs sometimes instruct neurogenesis, ciliary neurotrophic factor or BMPs instruct astrocytic differentiation, and thyroid hormone instructs oligodendrocyte differentiation (Li *et al*., 1998) studies have since demonstrated that by following a well-defined protocol, and by using EGF, basic fibroblast growth factor (bFGF), or both as mitogens, it was possible to produce a consistent, renewable source of undifferentiated CNS precursors, which could be expanded as neurospheres, or differentiated into defined proportions of neurons, astrocytes, and oligodendrocytes (Gritti *et al*. 1999)). Isolation and long-term culturing of primary stem/progenitor cells have been advanced by the findings that the mitogenic growth factors epidermal growth factor (EGF) and basic fibroblast growth factor (FGF-2) have proliferative effects on these cells (McKay, 1997) [30]. Adult rat hippocampus derived FGF-2 responsive cells grafted to homo- or heterotypic neurogenic sites (hippocampus or olfactory system) differentiated into neurons (Gage *et al*., 1995b; Suhonen *et al*., 1996) [43], whereas when they were grafted to non-neurogenic site (cerebellum) no neuronal differentiation was observed (Suhonen *et al*., 1996) [43]. In contrast, EGF-responsive embryonic mouse stem cells grafted in intact neonatal mouse cortex and spinal cord showed poor survival and no neuronal differentiation. The most commonly used methods for the isolation and culture of stem cells use serum-free culture medium supplemented with various hormones and nutrients (Bottenstein and Sato, 1979) [6] and mitogenic growth factors EGF or FGF-2. EGF has been used to culture subependymal/forebrain stem cells as neurospheres from embryonic and adult mouse (Reynolds *et al*., 1992) [40].

Numerous investigators have isolated and expanded neural stem/progenitor (precursor) cells *in vitro* using a variety of mitogens and culture conditions (Gage *et al*., 1995a) [14] Mitogenic growth factors, epidermal growth factor (EGF) and fibroblast growth factor (FGF-2), stimulate the proliferation of multipotent stem/ progenitor cells from embryonic and adult rodent, primate and human CNS (Gage *et al*., 1995b) a combination of EGF and FGF-2 is needed to culture embryonic and adult mouse spinal cord, striatum and subventricular zone (SVZ)-derived progenitor cells as neurospheres (Gritti *et al*., 1999). Although stem/progenitor cells from different species respond to growth factors, alone or in combination, their growth properties differ depending on conditions (Hitoshi *et al*., 2002) [21]. neurospheres, and are

kept proliferating by adding growth factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and/or leukemia inhibitory factor (LIF) (Palmer *et al*., 2001). Recent studies also signaling. FGF together with Wnt signaling regulates late features of the dorsal telencephalon (Gunhaga *et al*., 2003) [19]. Levels of active β-catenin are increased in neural stem cells from the ganglionic eminence when they are cultured in the presence of FGF2. Moreover, overexpression of β-catenin in the presence of FGF2 maintains neural progenitors in a proliferative state, while overexpression of β-catenin in the absence of FGF2 results in enhanced neuronal differentiation in adhesive cultures. In cortical cells, however, Wnt supports both maturation and proliferation, but only the maturation effect has been reported to be FGF-dependent (Viti *et al*.,2003).

Isolation and Culture of Neural stem cell

In the order to get the neural stem cell for culture or therapeutic uses, we have to isolate the cell from fetal brain and certain specified region of the adult brain which harbor the neural stem cells like hippocampus and ventricles. Long-term cell culture systems have been developed for rodent and human central nervous system (CNS) cells that continuously propagate a heterogeneous population of early neural stem or progenitor cells. Rao (1999) [39] isolated adherent cultures of E10.5 rat neuroepithelial cells ‘‘termed neuroepithelial (NEP) stem cells’’ from the caudal neural tube at early stages of development (Kalyani *et al*., 1997) [22], while most previous reports showed only more restricted precursors could be isolated as adherent culture on fibronectin and polyornithine (Ray *et al*.,1993; McKay, 1997) [30]. Reynolds *et al*. (1992) [40] isolated neural stem cells for the first time. Neural stem cells cultured from early to mid-gestation give rise to more neurons than those cultured at later periods, a property that appears to be cell-intrinsic (Qian *et al*., 2000) [38]. To obtain long-term proliferative cultures, cells from embryonic brains were immortalized by using oncogenic transgenes like v-myc or SV40 large T antigen (Whittemore and Snyder, 1996) [49]. The immortalization process arrests cells at specific stages of development and stops their terminal differentiation (Lendahl and McKay, 1990). Clonal cultures of cells representing a specific stage in development are isolated and used for *in vitro* and *in vivo* studies (Gage *et al*., 1995a; Fisher, 1997) [14, 12]. Although immortalized cells offer a number of advantages, they do not always represent their primary counterparts. Isolation and long-term culturing of primary stem/progenitor cells have been advanced by the findings that the mitogenic growth factors epidermal growth factor (EGF) and basic fibroblast growth factor (FGF-2) have proliferative effects on these cells (McKay, 1997) [30]. Isolated and cultured stem cells not only provide an important source of cells for *in vitro* studies to address issues related to fate choice and differentiation, but they are also an important source of CNS cells that can be used in transplantation studies (Ray *et al*., 1997, 1998).

Neuronal Differentiation

Chang *et al*. (2005), isolated and cultured the neural stem cells from the developing cerebral cortex of premature human infants using gradient-generating microfludic platform. Cells were cultured in medium consisting of Dulbecco’s modified Eagle’s medium: F12 nutrient mixtures supplemented with 1% antibiotic/ antimycotic and growth factors (epidermal growth factor, fibroblast growth factor 2 and platelet-derived growth factor). NSCs were cultured at different concentration growth factors using gradient chamber of the microfludic device.

Chen *et al*. (2010) isolated the NSC from mouse embryos at day 11.5 were collected from the pregnant strain mice using the media having the growth factors. basal medium (a 1:1 mixture of Dulbecco’s modified Eagle’s medium high glucose/Ham’s F12 medium containing 100 units/ml penicillin and 100 mg/ml streptomycin).

Villa *et al*. (2009) [86], cultured the NSC from forebrain, dorsal and ventral mesencephalic regions of a 10 week old aborted human foetus after informed consent of the women seeking abortion Cells were plated in 10 µg/ml poly-lysine pre-treated plastic ware, grown and differentiated at 37 °C in a 95% humidity, 5% CO2 and 5% O2 atmosphere. Cells expressed the NSC markers nestin, vimentin and stem cell marker sox 2.

Egawa *et al*. (2011) [88], isolated NSCs from rat using collagen hydrogel incorporating engineered epidermal growth factor. In the transplantation studies most of the cells died because of apoptosis and inflammatory factors. To overcome this problem, collagen-based hydrogels were designed to provide microenvironments for embedded cells to survive and proliferate. EGF as a mitogen, was fused with a collagen by recombinant DNA technology. Collagen is intrinsically inert for NSCs (Uemura *et al*.2010) [62].

Wang *et al*. (2011) [90], revealed that astrocytes secrete molecules which play important roles in the cell fate determination of neural stem cells. However, the exactmolecules involved and its possible mechanisms in the process remain largely unknown. The telencephalon was dissected out from the embryo and the cultures were incubated in the growth medium containing DMEM/F12 supplemented with basic fibroblast growth factor, epidermal growth factor and 2% B27 (growth medium). The cells were grown as floating neurospheres.

Hung and Young (2006) [91] studied the effect of fetal bovine serum in substrate-coated and soluble form on neural stem cells.They cultured the NSC which were isolated from cerebral cortex of rat.

They seeded the bFGF responsive neurospheres on EVAL (ethylene-co-vinyl alcohol) and PVA (Polyvinyl alcohol) substrates in order to study the effects of these substrates on the behavior of neural stem cells.

Lee *et al*. (2009) [92] investigated the effect of EGF and FGF on mouse derived neural stem cell/neural progenitor cells. Growth factor responsive cells had lesser proliferation rate and neurospheres forming capacity However, EGF/FGF1-responsive mouse brain cells had better neural differentiation capacities than EGF/FGF2-responsive NSPCs. Fibroblast growth factors have been shown to maintain the proliferation, self-renewal and multipotent capacities of neural stem/progenitor cells (NSPCs) *in vitro*. They characterized the cell by CD marker i.e. CD133.

Hook *et al*. (2011), described generation of adherent, homogeneous, non-immortalized mouse and human neural stem cells derived from both brain tissue and pluripotent embryonic stem cells for high quality uniform cell production. They generated more than 20 adherent hNS lines from whole brain, cortex, lobe, midbrain, hindbrain and spinal cord.These cells expressed the various marker such as CD 133, nestin, and maintained neurogenic and multipotential differentiation ability after extensive long-term expansion.

Xu *et al*. (2010), evaluated the possibility of PHA (Polyhydroxyalkanoates) nano-fiber matrices to promote

neural growth and differentiation. (PHA) have been demonstrated to be a family of biopolymers with good biodegradability and noncytotoxicity (Chen and wu, 2005).

Neuronal differentiation

It is very essential to differentiate the NSC into neuron as they are required in various neurological disorders. Neurological disorders don't respond to the chemotherapy and they require the replacement and regeneration of cells i.e. motor neuron. 3-hydroxyhexanoate (PHBHHx) supported NSC growth and differentiation.

Chen *et al*. (2010). Studied the neural differentiation of NSCs on ultra-nanocrystalline diamond (UNCD) and polystyrene The ECMs (extracellular matrix) have various effects in providing structure supports or regulating cell proliferation and differentiation.

Li *et al*. (2005) exploited differential potential of hsNSCs at different passages was analyzed by immuno fluorescent staining or real-time PCR. Neural induced conditions for hsNSCs had been mentioned before. IBMX (isobutylmethylxanthine), forskolin, bFGF, and dbcAMP (dibutyryl cyclic AMP) were added into the DMEM/F12 culture media containing 10% FBS for 3 days to induce neuron differentiation.

Villa *et al*. (2009) [86] removed the mitogen to poduce differentiated neural cell from 10 week old aborted human fetus. Differentiating media consist of DMEM/F12 supplemented with 10 μM dopamine (DA), 10 μM forskolin (FK) (Sigma), and 50 ng/ml BDNF (brain derived neurotrophic factor).

Corti *et al*. (2012) [87] showed that the Astro NANOG (Human astrocytes were transfected with Nanog)- derived cells exhibited a gene expression pattern similar to that of neuronal stem/precursor cells, To characterize neuronal differentiation capacity, Astro NSC clones were cultured in human neuronal differentiation medium.

Therapeutic potential of NSC

Neurological disorders, such as Parkinson's disease, stroke and multiple sclerosis, are caused by a loss of neurons and glial cells. Presently, neurons and glia have been generated successfully from stem cells in culture, making efforts to develop stem-cell-based transplantation therapies for human patients (Lindvall and Kokaia, 2006) [93]. In humans, the existence of NSCs has also been reported in embryonic and adult human brain with multipotent differentiation capability (Kim *et al*.2004). Cancer patients had infusion of bromodeoxyuridine (BrdU) for diagnostic purposes and later died, prove that new neurons are continuously being generated in adult human CNS has been demonstrated (Erkisson *et al*., 1998).

Parkinson's disease (PD) is a gradual loss of nigrostriatal dopamine-containing neurons, but degeneration also occurs in non-dopaminergic neurons. The main symptoms are rigidity, bradykinesia, tremor and postural instability. A stem-cell-based therapy must lead counteract disease progression. Clinical trials of the transplantation of human fetal dopaminergic neurons have shown that cell replacement can produce major, long-lasting improvement in some patients (Lindvall *et al*., 2004) [67]. Survival of the cells after transplantation in animal model has been poor. (Piccini, 2005) [68]

Stroke

Stroke occur due to blockage of a cerebral artery, resulting to focal ischaemia, loss of neurons and glial cells, and motor, sensory or cognitive impairments. Transplanted cells from different sources(fetal brain, neuroepithelial or teratocarcinoma cell lines, bone marrow and umbilical cord) have resulted in some improvement in animals and, in one clinical trial, in humans affected with stroke (Lindwall *et al*., 2004) [67]. The grafts have acted by providing trophic factors that enhance cell survival and function (Lindwall *et al*., 2004) [67]. Human fetal neural stem (NS) cells were transplanted into the brains of stroke-damaged rats, resulting in the migration of new neurons towards the ischaemic lesion (Kelly *et al*., 2004) [69]. Studies showed that monkey ES-cell transplanted into the brains of mice after stroke differentiated into various types of neuron and glial cell (Hayashi *et al*., 2006) [70], and led to improved motor function (Ikeda *et al*., 2005) [71].

Huntington's disease

Huntington's disease (HD) is a fatal disorder that is characterized by chorea and progressive dementia. It is caused by the loss of projection neurons in the striatum. In animal models of HD, cell replacement using grafts of fetal striatal neurons promotes functional recovery, and some evidence from clinical trials indicates that this can also occur in patients (Lindvall *et al*., 2006) [93]. However, human NS cells implanted into the brains of rats were found to reduce motor impairments in experimental HD (McBride *et al*., 2004) [73]

Amyotrophic lateral sclerosis

In amyotrophic lateral sclerosis, dysfunction and degeneration of motor neurons occur not only in the spinal cord but also in the cerebral cortex and brainstem. Muscle weakness progresses rapidly and death occurs within a few years. Recent reports have shown that it is possible to generate lower motor neurons *in vitro* from stem cells of various sources, including ES cells and those from the fetal CNS (Li *et al*., 2005). Mouse ES-cell-derived motor neuron sestablish functional synapses with muscle fibres *in vitro* (Miles, 2004) [83] and extend axons to ventral roots after transplantation into adult rats (Harper *et al*., 2004) [72].

Alzheimer's disease

Alzheimer's disease (AD) is characterized by neuronal and synaptic loss throughout the brain, involving the basal forebrain cholinergic system, amygdala, hippocampus and several cortical areas. No effective treatment is currently available except for acetylcholinesterase inhibitors, which alleviates cholinergic function, but this is only a temporary measure. Nerve growth factor (NGF) prevents neuronal death and improves memeory in animal models of aging, excitotoxicity, and amyloid toxicity (Tuszynski, 2002) [84]

Multiple sclerosis

Multiple sclerosis (MS) is caused by the destruction of the myelin sheath that surrounds axons, leading to conduction problem and neurological symptoms. Immunomodulatory and immunosuppressive treatments are only partially effective. Myelin-producing oligodendrocyte progenitor cells (OPCs) are abundant in the adult human brain (Windrem *et al*., 2004) [56]. An important area of research is that focused on finding ways to enhance remyelination from these cells, and identifying the causes that lead to a failure of cells to produce myelin. To this end, Back *et al.* (2005) [61] recently showed

that astrocyte-derived hyaluronan accumulated in demyelinated lesions from MS patients and prevented the maturation of endogenous OPCs. Human adult and ES-cell-derived OPCs have been shown to myelinate dysmyelinated mouse brain and spinal cord after transplantation.. Interestingly, after systemic administration in mice, NS cells migrated to inflammatory demyelinating lesions, where some became OPCs and remyelinated (Pluchino *et al*., 2003) [60].

Spinal cord lesions

Spinal cord injuries disturb ascending and descending axonal pathways, and cause a loss of neurons and glia, inflammation and demyelination. The lesions lead to a loss of movement, sensation and autonomic control. The transplantation of stem cells into injured spinal cord can lead to improvement (Ogawa *et al*., 2002), mainly through trophic factor secretion or there myelination of spared axons. A recent study showed that human NS cells implanted into damaged mouse spinal cord generated new neurons and oligodendrocytes, leading to locomotor recovery (Cummings *et al*.,2005) [57]. However, there are risks of side effects unless NS-cell differentiation after transplantation is controlled. Astrocytic differentiation and aberrant axonal sprouting after NS-cell implantation into injured rat spinal cord can cause hypersensitivity to stimuli that are not normally painful (Hofstetter *et al*.,2005) [58].

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