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Neural Stem Cell Approaches to CNS Repair

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Abstract

Neural stem cells (NSCs) can self-renew and give rise to neurons, astrocytes and oligodendrocytes. NSCs are found in the central nervous system (CNS) of mammalian organisms, and represent a promising resource for both fundamental research and CNS repair. Animal models of CNS damage have highlighted the potential benefit of NSC-based approaches. Here we review the progress in the field of NSC biology, including evidence of NSCs presence in the adult brain, NSC markers and current advances in NSC-based approaches for regenerative medicine.

Keywords: Neural stem cell, differentiation, developmental biology, regenerative medicine.

1. Introduction

1.1 Stem cell in regenerative medicine

Stem cells have self-renewing potential and the ability to produce differentiated derivatives de novo. These unique properties make them an ideal resource for regenerative medicine applications aiming to repair damaged tissues by supplying different types of cells, organs or tissues [1]. NSCs self-renew and give rise to neurons, astrocytes and oligodendrocytes [2, 3]. Stem cell-based approaches to tissue repair are in development for a number of therapeutic applications, in particular for orthopaedic treatment [1]. Studies carried out over the last decade have established the presence of neural stem cells in postnatal and adult stages, and highlighted the characteristics and differentiation potential of these neural progenitors. Neural stem cells (NSCs) represent potentials candidates for neural repair by replacement of lost or damaged CNS cells, and recent advances have opened new prospects for stem cell-based approaches to CNS repair.

1.2 Evidence of NSCs in the adult brain

Early work from Altman in the 60s presented autoradiographic and histological evidence of neurogenesis in the dentate gyrus (DG) of the hippocampus, neocortex and olfactory bulb of the adult rodents [4-6]. Many subsequent studies confirmed this observation [7, 8], and the use of techniques labelling dividing cells such as bromodeoxyuridine (BrdU) confirmed the occurrence of neurogenesis not only in adult rodent brains, but also in primates [9, 10]. Lois and Alvarez-Buylla showed that dividing cells labelled with [3H] thymidine in the SVZ can differentiate into neurons and glia *in vitro*, identifying the SVZ as a source of neural precursors in adult mammalian brain [11]. Using a similar approach, Corotto also found new cells are generated in the subependymal layer (SVZ), and migrate to the olfactory bulb, where the majority of the newly generated cells persist at least 16 weeks [12]. In adult primates, Kornack and Rakic reported new neuronal cells generated in the DG of hippocampus and the SVZ of adult macaque monkeys [10, 13] and in humans, Erisson presented the first evidence of neurogenesis in DG of the adult human brain and further indicated that the human hippocampus retains the ability to generate neurons throughout life [14]. More recently, Sanai found a group of SVZ astrocytes in the lateral ventricles of the adult human that proliferate *in vivo* and possess stem cell properties *in vitro*. Also, the study showed no evidence of neuronal migratory stream to the olfactory bulb in human brain, which is still in debate [15-17].

The persistence of neurogenic activity in the adult brain suggests the existence of NSCs [18]. Experiments have been developed to identify and characterise NSCs using the 2 criteria defining stem cells: - multipotency, i.e. the ability to generate both glial and neuronal cell types from a single cell, - self-renewal, i.e. the maintenance of stem cell characteristics over serial passages.

Neural progenitor cells have first been isolated from adult mouse brain tissue including SVZ. When isolated *in vitro*, these cells were shown to grow as floating colonies of progenitor cells, known as neurospheres [3] (Figure 1A). In 1995, Gage et al. isolated and characterisized progenitors from adult rat hippocampus, which also formed neurospheres and retained the capacity to generate mature glia

and neurons when transplanted into the adult rat brain [19]. Based on previous findings, Reynolds and Weiss developed a method to test the self-renewal potential of these progenitor populations by performing a clonal analysis. In this procedure, neurospheres are dissociated into single cells and plated in individual wells. Formation of clonally derived spheres maintaining the differentiation potential of the original culture over serial passages is used to assess the stem cell characteristics of the culture [20, 21].

1.3 Nature of NSCs

Doetsch et al. have shown that SVZ astrocytes labeled *in vivo* give rise to multipotent neurospheres *in vitro* [22], suggesting a model in which dividing SVZ astrocytes give rise to immature precursors, which themselves divide to form tight clusters that generate migrating neuroblasts. This model has been strongly supported by other studies [23, 24]. Using similar approach, Seri identified SGL astrocytes as NSCs in the adult hippocampus [25]. These hippocampal NSCs also share astrocytic characteristics including GFAP expression, and are able to divide and generate neurons under normal conditions or after treatment. Filippov et al. used the nestin-GFP transgenic mouse model to confirm that the putative NSCs in the adult hippocampus share astrocytic features [26]. Although some studies have reported the presence of NSCs among the non-GFAP expressing population of the adult SVZ based on flow cytometry [27, 28], the glial origin of NSCs appears largely accepted [9, 29].

1.4 NSCs present in other areas

Apart from the SVZ and the DG, other regions of the postnatal brain have been reported to contain NSC-like cells. Putative NSCs have been identified in the 7-day old mouse cerebellum of mice, where the cells were mainly located in the white matter [30]. Klein et al. reported the existence of putative NSCs from the adult cerebellum (P>42 days) using the neurosphere assay [31], although there is still a debate about the distribution and function of NSCs in the adult cerebellum [32, 33]. Putative NSCs have also been isolated from adult spinal cord, and throughout the entire ventricle neuroaxis of mice [34]. Clonal analysis demonstrated that these cell populations proliferate in response to EGF+bFGF, and share the ability to self-renew, expand and maintain their multipotency shown for SVZ NSCs [34]. NSCs isolated from the lateral, 3rd and 4th ventricle, as well as from the thoracic and lumbar/sacral segment of spinal cord were compared *in vitro*. The lumbar/sacral spinal cord presented the strongest neurosphere-forming ability, compared with the lateral, the third and fourth ventricles. However, only cells in the lateral ventricles could proliferate and expand in response of EGF alone [34].

Outside of the brain, Ahmad et al. reported a population of mitotically quiescent cells in the pigmented ciliary body of the adult rats with chronic BrdU injection. These cells also proliferated in the presence of FGF2, formed self-renewing neurospheres, and were able to differentiate into neurons and glia [35].

1.5 Markers for NSC characterisation

There isn't to date a single marker uniquely specific for the adult NSC. NSCs have been reported to express a range of markers such as those listed below, which, when detected in combination, are routinely used to identify this stem cell population [36].

Nestin is widely used to characterise NSCs [37, 38] (Figure 1B). This intermediate filament protein was found to be expressed predominantly in neuroepithelial stem cells during embryogenesis [39]. In differentiated cells, Nestin expression is sharply reduced and is replaced by tissue-specific intermediate filament protein [37, 39]. The use of the Nestin-GFP transgenic mouse, where GFP (green fluorescent protein) expression is controlled by the regulatory sequence of nestin, allows NSC isolation by FACS (fluorescent-activated cell sorting) based on GFP expression [40].

Musashi1 is a member of neural RNA-binding family used as a marker for neuroprogenitors [37, 41, 42]. In the developing rodent brain, Musashi1 is expressed predominantly in proliferating multipotent neural stem cells; however Musashi1 expression is reduced during neurogenesis and lost in newly generated post-mitotic neurons. Musashi1 is reported to have a role of maintaining the self-renewing ability of NSCs by positively regulating the Notch signaling pathway [43].

Sox1 and **Sox2** are members of the SoxB1 subfamily of transcription factors expressed in early embryonic neuroepithelium [44]. Sox1 expression is observed in proliferating neural precursors throughout embryogenesis and into adulthood [45]. Sox1 expression is down-regulated as progenitors exit from mitosis and differentiate into neurons or glia, suggesting a role in the maintenance of the undifferentiated state [46]. Sox2 is expressed in ES cells, in embryonic neuroepithelium stem cells and in neurogenic regions of the CNS throughout adulthood [47]. Sox2-positive neuroprogenitors express nestin, and SVZ-derived neurospheres lose Sox2 expression upon neuronal differentiation [48]. Sox2 is required for the maintenance of NSCs, as inhibition of Sox2 signaling is associated with neuronal differentiation whereas constitutive expression inhibits progenitor differentiation [49].

ABCG2 is a member of the ATP-binding cassette (ABC) family of transporter proteins present in many normal and malignant tissues. Cultured human neuroprogenitors express ABCG2, and the proportion of ABCG2-positive cells in neurospheres is similar to that of Nestin-positive

cells [50]. ABCG2 and Nestin have often been shown to co-localize in the same cells, and ABCG2 expression is sharply down regulated during differentiation [50].

Prominin-1 (also named CD133) is a transmembrane protein originally identified on hematopoietic stem cells [51, 52] before being used to isolate neuroprogenitors [52, 53]. FACS analysis has shown that CD133-positive cells from human fetal brain can form neurospheres, self-renew and show multipotency at the single-cell level [53]. Very recently, CD133 has been used to identify and isolate neuroprogenitors from embryonic and adult mouse brain and was found to be co-expressed with other NSC markers including Sox1, Sox2, Nestin and Musashi [54].

Integrins are cell surface receptors mediating interactions between cells and the extracellular matrix [55]. β1 integrin was found to be as effective as CD133 for NSCs isolation, and cells with high levels of β1 integrin expression also show increased expression of NSC markers such as CD133, nestin, sox2, and musashi1 [56].

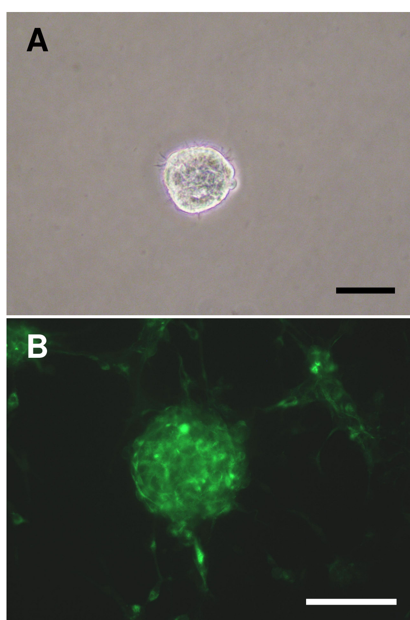

Figure 1. Neurosphere isolated from mouse SVZ tissue. (A) Phase contrast view of a single neurosphere. (B) Nestin immunostaining of SVZ neurosphere. Bar: 100µm.

2. Stem cell research for applications in regenerative medicine

Because of their proliferative and differentiation properties, NSCs represent a promising resource for future approaches aiming to repair damaged or lost brain cells in a range of pathologies. However, one of the main unresolved issues precluding such developments remains the availability of human NSCs suitable for cell therapy.

2.1 Human NSCs:

Human NSCs have been isolated from fetal and adult postmortem brains [57-59], mostly from SVZ tissue and other regions including hippocampus, white matter, olfactory bulb, cortex and spinal cord [60-65]. The dissected tissue is mechanically disaggregated, and the dissociated cells grow as neurospheres in a serum-free defined medium containing bFGF, leukemia inhibitory factor (LIF), and EGF. These cultures were shown to allow a 10^7 fold increase in the number of cells while retaining their differentiation potential towards neurons and glia [66-68]. Fetal and adult NSCs present many advantages for therapy purposes. NSCs are known to efficiently generate differentiated lineages [69], and to have the ability to migrate towards injury sites [70] where they may secrete factors [71] promoting proliferation and repair [72-74].

2.2 Embryonic stem (ES) cell-derived NSCs

Pluripotent ES cells can differentiate into multiple neural phenotypes in the presence of neurotrophic factor [75-81]. Several approaches have been taken to promote neuronal differentiation in ES cultures, including exposure to retinoic acid, low-density culture, growth factors treatment, co-culture with other cell types, and forced expression of lineage-specific genes [82-85]. Neural progenitors generated *in vitro* from both ES cells can be sorted from ES cultures using surface antigens [86]. Human ES cell-derived neuroprogenitors can integrate into the ventricles of newborn mice and differentiate [80]. ES cells are amenable to large scale culture and permissive to genetic modification enabling expression of therapeutic agents [69, 87]. However, ES cells have the ability to produce teratoma-like growths when injected into mice, thus presenting an inherent safety issue for any ES-derived therapeutic approach [80, 88].

2.3 NSCs from non-neural cells

Other adult stem cell populations have been reported to exhibit some neural potential *in vitro* and sometimes *in vivo*. Mesenchymal stem cells (MSCs) represent a bone marrow cell population able to differentiate into bone and cartilage derivatives [87, 89, 90]. It has also been suggested that bone marrow-derived stem cells can give rise to extra-mesodermal lineages including neural cells both *in vitro* and when transplanted into rodent models [91, 92]. Doubts remain on whether these observation may arise from transdifferentiation (direct change into neurons) [91-94], transdetermination (change into stem cells of different origin)[93], or cell fusion (integration of assimilated cells into existing neurons) [95].

MSCs present many advantages for cellular therapy, since they are easily accessible for collection [87] and allow autologous transplants without immunosupression issues [96-98]. MSCs can survive after implantation in the brain and migrate broadly [96, 99-101], however, it is still

unclear whether neural-like cells derived from such non-neural tissues would be able to respond appropriately to signals within the brain [87, 92, 102].

3. NSC applications

3.1 Targeted pathologies

The discovery of NSCs opens the possibility to develop future therapies to replace neurons damaged due to injury or neurodegenerative diseases through two approaches: exogenous NSCs transplantation, and endogenous NSC activation [103, 104]. NSCs-based application are envisaged around 2 main strategies: (i) cell replacement, as NSCs are delivered intracerebrally or intravenously and reach the target organ to generate appropriate cell types; (ii) neuroprotection, using NSCs as vehicles to provide neuroprotective molecules such as glial cell line-derived neurotrophic factor (GDNF) to the injury site [105]. Rodents provide critical *in vivo* models for studies evaluating the feasibility and efficiency of NSC-based applications for a range of conditions such as those highlighted below.

-Alzheimer's disease (AD) is characterized by progressive impairment of memory and cognitive functions [106] due to neuronal degeneration and synaptic loss throughout the hippocampus, neocortex, amygdale, thalamus and substantia nigra [107]. Cognitive decline caused by the progressive loss of differentiated cells could in theory be restored through transplantation of NSCs or NSC-derivatives [108]. As genetically modified NSCs have shown migration capacity after transplantation, they could serve as vehicles to deliver therapeutic molecules [108, 109].

-Huntington's disease (HD) is a fatal disorder characterized by degeneration of projection neurons in the striatum due to mutation of huntingtin gene [108]. The potential of a stem cell-based approach would be to preserve of brain function by replacing neurons in the striatum. In rat and monkey models of HD, the transplantation of fetal striatal tissue containing projection neurons has shown improvement in motor and cognitive functions [110-112]. Furthermore, clinical trials involving HD patients have demonstrated that human fetal striatal grafts can survive and integrate without typical pathology at least for 18 months [113]. It is therefore conceivable that NSC transplantation may similarly provide a possible alternative. Adult neural progenitors isolated from the SVZ and transplanted into striatum of a HD rat model were also shown to promote motor function recovery [114].

-Multiple sclerosis (MS) is an inflammatory and demyelinating disease characterized by the loss of the myelin sheath surrounding axons, resulting in signal conduction deficits and severe neurological symptoms [108]. Oligodendrocyte progenitors present are capable of producing myelin but do not remyelinate the axons affected [115]. One possible approach could be the transplantation of oligodendrocyte progenitors able to mature and myelinate *in situ* [116, 117]. One study reported that adult mouse NSCs isolated from the periventricular region forebrain transplanted into a mouse model of MS caused an increase in oligodendrocyte progenitors, some of which were found to actively remyelinate axons [118].

-Parkinson disease (PD) is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra, causing tremor and impairing movement [108, 109]. Early trials based on intrastriatal transplantation of human fetal mesencephalic tissue provided promising results in treated patients [119, 120], thus suggesting possible benefits for cell replacement strategies. In addition, using human stem cell for the delivery of neuroprotective molecules may help to hinder disease progression. Engineered human neural progenitor containing GDNF were transplanted into of rat and monkey striatum, and achieved cytokine release, which increased the survival and function of dopamine neurons [121].

-Spinal cord injures lead to loss of motor function, and stem cell transplantation into injured spinal cord has shown benefits in rats [122, 123]. Human NSCs isolated from fetal brain tissue using surface antigens [53] were transplanted into injured mouse spinal cord and gave rise to new neurons and oligodendrocytes, which promote locomotor recovery [124]. However, the differentiation of NSCs needs to be controlled to avoid abnormal axon sprouting leading to severe side-effects [125]. Using NSCs to improve remylination might represent a feasible approach [108], since there appears to be a correlation between the number of newly born oligodendrocytes from NSCs implants and the extent of locomotor function recovery in rats [125]. In another study, transplantation of human ES cell-derived oligodendrocyte progenitors into the injured rat spinal cord caused enhanced myelination and motor function improvement [126], thus highlighting the potential of stem cell-based approaches.

-Stroke results from interruption of cerebral blood flow producing ischemia, cell degeneration and long-term damages [108, 109]. One study describing the transplantation of human fetal NSCs into a rat model reported survival of the implanted NSCs and migration of newly formed cells towards ischemic sites [127]. Transplantation of primate ES cell-derived cells into a mouse model of stroke showed generation of neuronal and glial cells, restoration of connections and motor function recovery [128, 129]. A recent study in adult rats reported that SVZ NSCs could similarly contribute to generate neuroblasts which could differentiate into mature neurons able to migrate towards the ischemic damage [130].

4. Perspectives

Present in the CNS throughout postnatal life, endogenous NSCs represent a promising resource for brain repair. However, the number and regenerative capacity of endogenous precursors may represent limited therapeutic potential without some form of activation, particularly in cases of neurological disease [101]. In order to efficiently manipulate endogenous NSCs for therapy, the mechanisms regulating NSCs fate choice and proliferation need to be elucidated.

Activation of endogenous progenitors has been tested by intracerebroventricular infusion of EGF and bFGF into the lateral ventricle of a rat model, leading to the increased neurogenesis after bFGF treatment, and increased astrocyte production after EGF treatment [131]. In a rat model of PD, infusion of transforming growth factor- lead to proliferation toward injection sites [132]. Other exogenous molecules may also support activation of endogenous NSCs, such as Sonic hedgehog (Shh), which is promotes cell proliferation in the SVZ and SGZ [59, 133-135]. Such approaches thus represent important avenues for fundamental research, and may identify new targets for therapeutic strategies.

Over the last decades, research on NSCs has made rapid progress, providing tools for the identification and isolation of NSCs from both in embryos and discrete regions of the adult brain. Their therapeutic potential is under investigation using experimental models of CNS disease, and early results obtained in animal models suggest NSC-based treatments may provide functional benefit. However, many issues remain to be clarified: the ideal cell source for therapy, appropriate procedures for the *in vitro* manipulation to obtain the number of cells required for transplant, and cell delivery strategies. Moreover, a better understanding of the regulation of endogenous NSCs proliferation and differentiation in both pathological conditions and normal conditions could provide new means of non-invasive NSCs therapy, through directed recruitment and differentiation of the desired cell type in situ in order to replace cells lost by injury or disease.

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