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The Role of Neural Stem Cells in Neurorestoration

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1. Introduction

Many neurological diseases have a poor prognosis. Most neurological treatment is primarily based on minimizing secondary - or further damage – and to optimize the remaining neurological function. Even a highly successful treatment like deep brain stimulation for Parkinson's disease improves neurological function through conditional lesioning. Several neurodegenerative diseases have no established treatments[1].

The complex electrochemical, molecular and anatomical structure of the central nervous system is established during prenatal and early postnatal development. Thus, it was long considered impossible to heal or substitute destroyed nervous tissue. The adult human brain used to be viewed as static, as it was a common perception that no new neurons could be generated after birth. This has been referred to as the "no new neurons"-dogma[2], and it goes back to the early neuronanatomist and Nobel Prize laureate Santiago Ramon y Cajal, who stated that "nothing may regenerate in the brain or central nervous system, everything may die"[3]. This axiom was challenged in the 1960s, but the work by Joseph Altman and co-workers was met with skepticism and was generally not accepted by the scientific community[4, 5].

During the 1970`s and 80`s Fernando Nottebohm and his colleagues made some very important discoveries. They found that the vocal centers in the brain of male canaries increase in size prior to the breeding season when vocal activities escalate to play pivotal roles in mating. In a series of studies they found no proliferation in the vocal centers, but showed that cell divisions took place in the ventricular wall. The newborn neurons then migrated to the vocal centers where they were integrated in neuronal circuits[6].

Evidence for neurogenesis in the mammalian brain was first presented by Reynolds and Weiss in 1992. They isolated cells from the striatum of adult mice and induced proliferation by epidermal growth factor[7]. Subsequently subsets of the cells developed the morphology and antigenic properties of neurons and astrocytes. Some of the newly generated cells also expressed immunoreactivity for the neurotransmitters typically found in that area of the adult mouse brain. In 1998 Eriksson et al. identified cells with stem cell characteristics *in situ* in the brain of adult humans post mortem[37, 152].

Through a steadily improving knowledge, primarily over the last 20 years, we have found that the central nervous system harbors cells with the ability to divide, mature and restore function after damage. Through manipulation it is even possible to differentiate cells derived from other organs into functioning neural cells that could be used as treatments.

A new approach, based on regeneration of central nervous tissue, might allow for better treatments for several of these devastating diseases. Although awaited with great hope, the translation of this basic research into tested treatments for patients is still wanting.

2. Definition of neural stem cells

Stem cells (SC) can loosely be described as cells that (I) have capacity for self-renewal (symmetric division), and (II) can give rise to cells other than themselves through asymmetric cell division[8]. SCs give rise to more differentiated progeny; **progenitor cells**. These cells have a more restricted ability for proliferation and differentiation.

The development from a multipotent stem cell to a variety of differentiated progeny has been most thoroughly examined in the hematopoietic system[9]. Here a detailed set of surface markers and transcription factors has been described to identify stem cells and different subsets of progenitor and differentiated cells[10]. Such a molecular phenotyping of the hierarchical organization allows for a detailed functional description, and to form hypothesis readily testable. However, even in this relatively well characterized cellular hierarchy controversies exist both on the stem cell nature and on the correct phenotype of such cells.

Cells with SC characteristics that can give rise to neural tissue **or** are derived from the central nervous system (CNS) are called neural stem cells (NSC). NSC can be derived from several sources. In principal such cells can be classified according to the sources of origin. Cells can be isolated from embryos, fetal, or the adult CNS. Neural stem cells are multipotent, giving rise to the three major cell types of the mammalian CNS: neurons, astrocytes and oligodendrocytes. Adult stem cells, also referred to as somatic stem cells, are undifferentiated cells found among mature and specialized cells in a tissue or organ, and reside in various tissues in the human body, including the central nervous system. It is the stem cells of the adult brain that drive adult neurogenesis.

The hierarchy of somatic stem cell differentiation in solid tissue is however much less clear[11, 12]. In addition, little is known about the differentiation pathways from such stem cells into the main groups of cells comprising brain stroma. Suggested progenitor cell phenotypes may differ between different parts of the brain[13, 14]. The fact that there exist thousands of different types of neurons in the CNS adds magnitudes of complexity. The impact of *in vitro* cultural artifacts confuses available data even further. Similar problems of stem- and progenitor-cell identification are present in several other organ systems where somatic stem cells have been described (breast, lung, prostate, skin, and gut). With such an uncharted landscape, defining a definitive SC population clearly poses a great problem.

Several approaches have been used to isolate and identify potential NSC. After the successful use of flow cytometry for identification of SC in the hematopoietic system, surface markers have been sought for NSC. The marker CD133 (also termed prominin-1 or AC133) was initially identified on a subset of human hematopoietic stem and progenitor

cells [15]. Antibodies directed at this protein were shown to prospectively identify a population of progenitor cells isolated from fetal human brain tissue[16]. CD133 is also expressed by the slow-dividing fraction of human umbilical cord progenitor cells[17]. This marker has been identified in the subventricular zone (SVZ) and rostral migratory stream [18] and even cortex [19] in human post-mortem derived tissue. Conflicting data exist however[20, 21], where a group identified CD133 positive cells in cells derived from embryonic SC, ependymal cells and brain tumor cells – but not in neurogenic cells derived from the adult human subventricular zone. This discrepancy may be due to technical issues, but could also be related to the plasticity of these cells in vitro as CD133 levels seems to be affected by bioenergetic stress[22-24]. Due to the discrepancy between studies, other adult human neural stem cell (ahNSC) or precursor markers have been suggested (SSEA1, CXCR4, A2B5, peanut-agglutinin ++)[21, 25]. These are less explored, but all seem to struggle with the level of variability and heterogeneity.

SCs are more robust than differentiated cells. The fact that NSC can be isolated from human brain >48 hrs post mortem exemplifies this fact[26]. Another well known example is the regrowth of hair lost during chemotherapy treatment. During chemotherapy patients loose hair one to three weeks after initiation of therapy. However, the SC of hair follicles survive, and usually hair grow back from three to six months after termination of therapy[27]. The molecular machinery behind increased DNA-repair mechanisms, free-radical scavengers systems and membrane pumps to expel toxic substances have been described in a range of cancers[28]. The presence of the same molecular machinery in a variety of malignancies implies that such mechanisms are based on activation of intrinsic cellular properties and signaling events. The molecular machinery allowing protection of somatic stem cells could be used to prospectively identify and enrich for such cells. The efflux of toxic substances by ABC (ATP Binding Cassette Transporter) membrane pumps was used to identify a population of cells with high efflux of the DNA-binding dye Hoechst 33342 with stem cell properties in murine hematopoietic system [29]. This functional phenotype was identified in fractions of cells isolated from developing mouse brain [30] and brain tumor cell lines [31]. Similarly, the ability to metabolize aldehydes has been used to identify stem cells in developing and adult murine brain[32]. Whether this approach will overcome the problems described above for surface markers is still unknown.

A third approach is to enrich for stem cells using culturing conditions selectively allowing for these cells to proliferate. This has been shown to effectively allow NSC proliferation in a range of species (murine[33], canine[34], porcine[35], monkey[36] and human[37]). Similarly, non-adherent, serum-poor culturing conditions have been shown to be applicable for SC in colon[38, 39], breast[40, 41], prostate[42,43], heart[44], skin[45, 46], pancreas[47, 48], and liver[49]. Under these conditions SC can proliferate extensively, while cells lacking this ability are eliminated. The demonstration of extensive self-renewal and generation of differentiated progeny by a large number of groups have shown this to be a robust method of isolating SC.

3. Neurogenesis and biology of endogenous NSCs

3.1 Neurogenesis and neurogenic regions

Stem cells differentiating into neurons (neurogenesis) have been identified in both the dentate gyrus of the hippocampus and in the walls of the lateral ventricles in the

subventricular zone (SVZ) and the rostral migratory stream (RMS) - the main pathway by which newly born neurons from SVZ reach the olfactory bulb. Cells in both neurgenic niches seem to translate through similar cellular development, but the anatomical organization is quite different.

In the dentate gyrus cells migrate only a few micrometers, from the subgranular zone to the granule cell layer. Cells develop from a precursor cell type in which mitotic events are found. Most of the newly formed cells are eliminated, and only few cells are able to establish axons, dendrites and functional synapses (postmitotic maturation phase). During the late survival phase characteristic electrophysiological patterns develop, receiving glutamatergic input from the entorhinal cortex and sending out axons to the hippocampal CA3 region. After a maturation period of several weeks the newly developed neurons establish characteristics identical to the other preexisting neurons[2].

The SVZ, in the walls of the lateral ventricles, contains the largest concentration of dividing cells in the adult mammalian brain[4, 50]. In the human brain there seems to be far more proliferating cells in the SVZ compared to the hippocampus[51, 52]. The cellular composition and organization of this region differs somewhat amongst species[53, 54]. In mammals the SVZ contains three cell populations important for stem cell proliferation. The proper stem cell population is maintained through slowly dividing astrocyte-like neural stem cells known as type B cells. These cells give rise to actively proliferating type C cells, which in turn give rise to immature neuroblasts, called type A cells. These neuroblasts, not yet neuronally committed, migrate to the olfactory bulb via chain migration by cell-cell contacts. Neuroblast chains are ensheathed by the processes of type B cells. In the anterior and dorsal SVZ, these chains condense to form the RMS [55-57]. After reaching the olfactory bulb cells migrate radially along blood vessel, and differentiate into interneurons incorporated into the functional circuitry of olfactory bulb and forebrain[50, 57].

In the adult brain, rodent and human studies reveal that neurogenesis continues in the SVZ throughout adult life[4, 56, 58-60]. The SVZ-RMS structure of the human brain contains 10^5 dividing cells, a number that is high compared with the rodent[51, 61]. As age increases in rodents, the number of neurogenic cells decreases[62, 63]. Early data based on magnetic resonance spectroscopy suggests that this may also be the case in humans[64].

Under normal circumstances the function of the SVZ is to produce neuroblasts for the RMS[51, 53, 56]. More recent experiments have demonstrated that the progenitors of the SVZ are capable of producing oligodendrocytes in addition to olfactory interneurons[65]. After experimental injury in animal models of Huntigton disease and stroke, the SVZ not only supplies the RMS with neuroblasts but SVZ progenitor cells also migrate toward the site of injury and cell death[66, 67]. Thus, the proliferation and migration from the SVZ responds to injury, suggesting a more important role for this region in neurorestoration.

3.2 Regulatory signaling of the NSC pool

The proliferation and differentiation of the NSC pool is highly regulated. The microenvironment maintaining this function is called the stem cell niche. This is a combination of signaling through extracellular matrix (ECM), cell-cell contacts, secreted substances, innervation and physical factors.

The niche is embedded in extensions of the vascular basal lamina that extends around NSCs and progenitors[68]. These laminin and collagen I-rich ECM structures can be observed under the electron microscope and have been named fractones. These structures has been suggested to bind secreted growth factors, like Fiborblast growth factor (FGF), regulating concentrations and signaling strength of secreted factors[69, 70], tenascin-C[70-72], osteopentin[73], chondroitin/dermatan sulfate proteoglycans[74, 75].

Ependymal cells, lining the ventricles, exert a supporting/ regulatory function in the niche, since they can modulate the transport of ions and other factors from the cerebrospinal fluid (CSF)[76]. They secrete neurogenic factors like pigment epithelium-derived factor (PEDF)[77] and the pro-neurogenic bone morphogenic protein (BMP) signaling substances[78, 79]. These cells also form gap junctions with SVZ astrocytes[80], allowing controlled transfer of substances from the CSF to the niche. NSC adapt close contacts to blood vessels both in the subgranular zone and the SVZ[69, 81]. This connection is suggested to be central in neurogenesis[82]. This could be through cell-cell-contact mediated signaling or through secreted factors like PEDF, leukemia-inhibitory factor (LIF) and brain-derived neurotrophic factor (BDNF) [83].

Several studies have shown effect on SVZ progenitor proliferation through infusion of growth factors into the ventricles. FGF, epidermal growth factor (EGF) and transforming growth factor alpha (TGFalpha)[62, 84, 85] have no identified source within the niche, but may originate from the choroid plexus and transported through CSF. Platelet derived growth factor (PDGF), PEDF and Vascular endothelial growth factor (VEGF) derived from endothelial cells regulate NSC and progenitor proliferation[77, 86-88], and PDGF also have effects on the differentiational balance between neurons and oligodendrocytes[86]. Several other secreted factors contributes to this orchestra of regulation like LIF[87, 89], BDNF[90, 91] and BMPs[78, 92].

Of special interest are the three stem cell related signaling pathways; Hedgehog-, Wnt- and Notch- pathways. Sonic hedgehog (Shh) is a morphogen known to regulate neurogenesis and gliogenesis during development. This signaling increase precursor and NSC proliferation both in the hippocampus and the SVZ[94-96] and Shh is essential for their maintenance[97]. Genetic manipulation by knocking-down the Shh signaling results in depletion of SVZ neurogenesis, while increased signaling leads to upregulation of proliferation[98]. Wnt-pathway signaling is orchestrated through a number of secreted Wnt ligands and a range of Frizzled receptors, and their interaction mediates the possibility for fine tuning of a proliferation-differentiation signal[99-102]. The combination of FGF and b-catenin signaling might be a requisite for neuronal differentiation[103]. Notch signaling is based on binding of ligands and receptors that are membrane bound, and thus acts through cell-cell interaction. This signaling is essential for niche maintenance, and again regulates both the size of the NSC pool and differentiation[104, 105], and differences in Notch signaling distinguish NSC from progenitors[106].

The convergence of synaptic input by classical neurotransmitters like γ-amino-butyric acid (GABA) and serotonin (5-HT) modulates the NSC niche. GABA is the principle inhibitory neurotransmitter in the adult CNS but has an excitatory action in the SVZ and the subgranular zone of the hippocampus[107, 108]. This effect is similar to its effect during brain development [109]. Isolated rat neuroblasts also express the GABA-A receptor. GABA has been found to decrease neuroblast migration[110] and to cause cell cycle exit[111], suggesting that the

number of dividing neuroblasts could be regulated by a feedback loop between NSCs and neuroblasts[112]. Major focus has been put on the serotonergic systems effect on the niche due to its importance in psychiatric diseases [113]. Early studies depleting serotonin (5-HT) in prenatal stages showed a reduction in cell proliferation in both neurogenic niches[114]. The effects of 5-HT are mediated on receptor level on NSC population might, however, differ in the SVZ and the subgranular layer[115, 116].

In Huntington's disease (HD) the SVZ increases in size, and has increased number of progenitor cells, while the mature cells present are altered. In Parkinson disease, on the other hand, the number of proliferating progenitors is almost halved compared to the normal situation[66, 67, 117-119]. This is believed to be related to the loss of dopamine stimulation of NSC proliferation.

Gas composition also affects NSC regulation. Processes of nitrergic neurons intercalate with neuroblasts in the SVZ [120]. Inhibitors of Nitirc oxide (NO) signaling affects cell proliferation and NO synthase deficient mice also exhibit higher levels of proliferation in the SVZ[120-122]. Oxygen tension highly affects the potency and proliferative potential of NSC[123, 124], and can switch the neurogenesis from differentiation of GABA-positive to glutamate positive neurons[125].

3.3 Cancer stem cells and their relation to NSC

The phenotype of neural stem cells is mirrored in several aspects of malignant tumor biology[126-128]. Several of the intrinsic molecular pathways and extracellular signaling systems identified in regulation of NSC have also been identified in cancer cells. Such cells, termed cancer stem cells (CSC) have been suggested to be essential in tumor growth and therapy resistance. Since NSC harbor the molecular machinery to respond to signals of proliferation and defense mechanisms to extrude toxic substances[129, 130], it has been suggested that NSC are the cell of origin for brain neoplasms[131]. By using conditionally targeted gene knock down of the tumor suppressor p53 in neural progenitor cells (Nestin +) and astrocytes (GFAP+), it has been demonstrated that both populations of cells can give rise to tumors[132, 133]. The induction of tumors however seem to be at lesser threshold by RAS and AKT transformation in Nestin+ cells, suggesting greater risk of tumor development from less differentiated cells. Similarly, different cell populations of NSCs, neural progenitor cells (NPCs) and more differentiated cells can all be candidates for malignant transformation[131]. In two subgroups of medulloblastomas different cells of origin and different molecular pathways seem to be important in tumorigenesis. Midline medulloblastomas present in the brain stem seem to develop from dorsal brainstem progenitors and be dependent on the Wnt- pathway. More laterally situated, cerebellar tumors seem to develop from granule neuron progenitors and be stimulated through SHH-pathway signaling[134].

The NSC pool and niche is highly controlled through a range of factors, underscoring the biological importance of these cell populations. Manipulating the signaling pathways for NSC homeostasis could thus be potential therapeutic intervention in brain tumors. Conversely, it is apparent that molecular signals or drugs that induce NSC proliferation could potentially be tumorigenic.

4. Challenges for the generation of NSCs

Several stem cell types have neural capabilities: 1. pluripotent self-renewing embryonic stem cells, 2. multipotent stem cells with broad potential and self-renewing capacity from embryonic, fetal or adult brain, 3. neural progenitors with limited potential and self-renewal capacity from adult brain or spinal cord, 4. committed neural progenitors (neuronal and glial) from brain subregions [135].

Embryonic stem cells (ESCs) have an almost unlimited capacity to self-renew. On the other hand, ESCs also have a considerable teratogenic potential after implantation into host tissue, and it is not yet clear how long chromosomal stability can be maintained[136]. In addition, immense ethical concerns exist regarding the use of human ESCs as well as government restrictions that continue to limit clinical applications [137].

Human fetal mesencephalic NSCs fulfill some important requirements for the use in cell replacement strategies. They can be generate high yields of functional neurons from a small starting population, representing on-demand availability of cells without major logistical problems and the possibility to standardize the cell source in a clinical setting. In contrast to ES cells, tumorigenicity seems to be a minor problem with fetal NSCs. These cells are less flexible with regards to multiplication and differentiation, but there is increasing evidence that it is more beneficial to use cells that are already committed to becoming a particular cell type[138].

More recently, induced pluripotent stem cells (iPS) were generated, and such cells offer another source of autologous neural stem cells[137]. It has been known that differentiated cells can be reprogrammed to an embryonic-like state by nuclear transfer to oocytes, fusion with ES cells or molecular reprogramming of somatic cells into induced pluripotent stem cells using genetic factors[138]. Most of the current reprogramming methods are using expression of putative oncogenes by retroviral vectors. The factors used are involved in carcinogenesis, posing a risk for clinical translation. Important questions regarding safety and genetic stability must be solved before iPS can be used in clinical trials[139].

Brain-derived ahNSC are very attractive because of the clear logistical benefits if the therapeutic stem cells can be derived from a patient's own body. Technical obstacles (obtaining fetal and embryonic tissue, immune graft rejection in hetero- and xenotransplantation, potential tumor formation after grafting of induced pluripotent cells) as well as ethical issues (in contrast to embryonic, fetal, hetero- or xenotransplantations of cells) can be avoided. Despite this, there are limited data concerning the application of adult human-derived neural stem cells in clinical trials and very limited number of experimental data[140].

Adult human neural stem cells can be isolated from a range of sources. Cells derived from the two neurogenic regions of the brain have been the most thoroughly examined, but cells with neurogenic potential in vitro can be derived from subcortical white matter[141], spinal cord[142], filum terminale[143, 144] and hypothalamus[145]. Also cells derived from the olfactory mucosa, found in the nasal cavity, contain ahNSC[146]. Several of these regions allow for harvesting of autologous NSC with minimal risk and morbidity for the patient[143, 146, 147].

Multipotent adult stem cells have also significant advantages with regard to autologous transplantation approaches without immunological graft rejection. Hematopoietic stem cells

(HSCs) and mesenchymal stem cells (MSCs) are valuable sources for cell transplantation and cell therapy. Although recent *in vitro* as well as *in vivo* studies suggested that multipotent adult stem cells, or their pro-neurally converted derivatives, could display protective or regenerative effects in experimental models of CNS diseases[138], more experimental data to translate the application of this type of cells to clinical trial is needed[137].

The discovery of multipotent stem and progenitor cells in the adult human brain has opened the possibility of treating central nervous system disorders through replacement of the injured tissue by transplanted cells or by stimulating recruitment of endogenous repair mechanisms. We have previously shown that in principle adult human neural progenitor cells (ahNPCs) could be transplanted to ischemically damaged brain for in vivo maturation into neurons[93,148]. The latter can be achieved both by infusion of growth factors or by transplanting progenitors delivering neurogenic factors to the injured brain. .

To obtain such a goal, one must have culturing protocols with the ability to obtain enough cells resulting in a clinically significant effect in one or more patients. In addition, the cells must survive long enough for quality testing and possible genetic manipulation before transplantation. One of the main obstacles when culturing ahNPCs has been that the cells seem to stop proliferating after a limited number of passages and also lose their ability for proper differentiation with repetitive passages[151]

The problem may however not apply to all ahNSC, as olfactory mucosa derived SC show higher propencity for proliferation and have been shown to be effective in an animal model of PD[195]. Also, it has been reported that ahNPCs can be propagated *in vitro* for as long as 20 months (12 to 15 passages) and have shown differentiation into cells expressing neuronal and astrocytic markers[149]. Together with a publication by Walton et al.[150], this article provides further evidence that the limitations upon continued propagation of ahNPCs previously reported by others may be surmounted.

Finally, when an adequate number of cells have been produced in vitro, the cells must be documented to have the appropriate ability to differentiate into mature neurons with the ability to produce synapses and generate functional action potentials. While we have documented this in cells cultivated short term in vitro[151-153], similar data on long term cultivated cells are lacking. We are looking forward to future experiments we hope will evaluate the ability of these long-term propagated progenitors for normal functional differentiation in vitro and in vivo.

5. NSC treatment strategies

Concerning the techniques of NSC application, regardless of the cell source, there are several treatment strategies that are explored in restorative approach.

5.1 Stimulation of endogenous NSC

It is evident that the adult brain contains a pool of NSC that have the ability to proliferate- and that can respond to extrinsic signals[154, 155].

Recent data suggests that NSCs and NPCs can migrate from their site of birth to other parts of the brain and contribute to the replacement of specific cell types lost due to injury or

disease[156-158]. In animal stroke models striatal neurons can be derived from endogenous NSC and progenitors [157, 159-161]. Similarly, compensatory neurogenesis exists in Huntington's and Alzheimer's disease patients. Compensatory neocortical neuron production have been demonstrated after targeted ablation of both interneurons and corticospinal neurons[162-165]. This neurogenesis is, however, quite modest and not associated with clinically significant functional effects. This is probably due to the limited number of stem cells recruited and/or the unfavorable environment of the injured adult brain for supporting efficient production of new neurons and glia.

Thus, the current challenge is to understand how to modify the molecular basis of compensatory neurogenesis in order to overcome its limiting factors in the pathological and aged CNS, while supporting those that accentuate its' influence.

Several of the described factors that affects the NSC pool are potentially tumor inducing when administered systemically, thus a major obstacle in developing this type of therapy is how to deliver the factor- or rather- the sequence of factors needed at high temporal and anatomical precision. Animal models have primarily used intraventricular injections or viral delivery methods to achieve this. Intraventricular injection of TGFAlpha activates endogenous neurogenesis in the SVZ of Parkinson's disease (PD) model rats [166, 167]. Similarly, the injection of the Notch receptor ligand angiopoietin2 or DII4 growth factors can induce widespread stimulation of endogenous neural precursors, and in a PD rodent model rescue injured dopamine neurons and stimulate improvement of motor function. Adenoviral co-delivery of BDNF and BMP signaling molecule Noggin induces striatal neuron replacement from endogenous precursors and delays motor impairment in a Huntington's disease model[168]. Intraventricluar injection of EGF and erythropoietin in combination can mobilize endogenous adult neural stem cells to promote cortical tissue re-growth and functional recovery after stroke[169]. Systemic erythropoietin is already in clinical use for the stimulation of erythropoiesis, thus allowing a rapid translation of this approach to clinical investigation. In a combination with the neurotrophic hormone β-human chorionic gonadotropin (hCG) this was found to be safe, and potentially beneficial in a phase II trial for the stimulation of neurogenesis after stroke[170].

5.2 Cell replacement by transplantation

As several obstacles remain regarding how to stimulate the correct cells with the correct sequence of stimulatory factors within the complex NSC niche, most therapeutic strategies are based on the transplantation of in vitro or ex vivo manipulated cells.

Most groups have favored the transplantation of immature cells. The idea is to let grafted cells differentiate under the influence of the host environment, integrate into the local neuronal network and thus become a functional unit of the brain or spinal cord. Immature cells are believed to be more robust than differentiated cells, and could contain the necessary plasticity to overcome pathological scar formation and inhibitory signals of relocation and differentiation. This approach is the most common in animal models of neurorestoration[2]. Also, the transplanted cells must have the ability to form the correct cells needed, and must stop proliferation when the proper cell types have been formed.

Better control of the developed progeny could be achieved by grafting of mature or at least partly differentiated cells. It is supposed that predifferentiation may help the processes of

functional integration of transplanted cells. We have shown that in selective injury of hippocampal CA1 region by global ischemia both ahNSC and predifferentiated cells preferentially migrate into the damaged area[93,148]. The predifferentiated cells develop more markers of differentiated neurons at an earlier time point. Thus, ahNSC can be manipulated *in vitro* to yield a greater neuronal differentiation after transplantation. In approaches where potential tumor forming cells are used, a controlled differentiation could reduce the risk of adverse tumor formation[165]. Similar in-vitro predifferentiation has been tested for generation of dopaminergic neurons in PD[169]. Further modification of the transplanted cells could be genetically manipulated cells that secret anti-apoptotic or pro-differentiation signal or a combination of NSC and stromal cells.

5.3 Microenvironmental modification

A third approach facilitates the ability of transplanted cells to affect the environment which the cells are transplanted into. Autocrine and paracrine factors derived from NSC can modulate the niche and stem-, progenitor and differentiated cells after transplantation. In rats it has been found that secreted growth factors from transplanted NSCs stimulated proliferation of endogenous NSC[171], called "bystander effect". In several transplantation studies functional recovery is far greater than the number of identified transplanted cells would indicate. This has been suggested to be a result of synergistic effects of the NSC on the host microenvironment.

Furthermore, transplanted NSC can secrete factors not present in the host. Infantile neuronal ceroid lipofuscinosis is a fatal neurodegenerative disease caused by a deficiency in the lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1). The lack of this enzyme leads to pathological lipofuscin-like material accumulating in cells, leading to progressive loss of vision, decreasing cognitive and motor skills, epileptic seizures and premature death. Normally functioning cells produce surplus of this enzyme, and some of this is secreted to the extracellular environment. This secreted enzyme can be absorbed by other cells, also cells not producing this enzyme on their own. This can be done in quantities high enough to stop lysosomal sequestering. In a mouse model lacking the gene for PPT1 transplanted NSC could reduce lipofuscine levels, provide neuroprotection and delay loss of motor function [172].

6. Towards using NSC to treat neurological disorders

Although NSC therapy have been suggested as a therapy for a range of neurological diseases, here we highlight the results for the most studied diseases; PD, stroke, and spinal cord injury.

6.1 Parkinson's disease

Over the past 30 years, neural transplantation has emerged as a possible therapy for PD. It was shown that grafted neural cells from different sources can survive for over 20 years and exert beneficial effects in PD patients[173]. Different types of cell have been tested both in experimental and clinical trial. Embryonic derived stem cells have been suggested the cell of choice, since they promise to be made in high quantities and to hold large amounts of the desired cell type [138]. Clinical testing of transplants to patients with PD of primary human embryonic dopaminergic neurons or tissue using double-blind, placebo-controlled protocols

have shown positive results. The patients displayed impressive improvements of symptoms and restoration of dopaminergic neurotransmission, but also demonstrated several clinical limitations. Only subpopulations of patients showed significant clinical benefits. Moreover, a significant proportion of patients (with up to 56%) suffered from dyskinesias after a twelve-hour drug-free period[174-176].

Whether, these early results could be transferable to the use of ESC is uncertain. Also, the use of ÈSC harbors problems of controlling cell growth and differentiation, including brain tumor and teratoma formation[138, 177-179]. In contrast, there are no reports of tumor formation in fetal NSCs transplantations, what makes the usage of fetal tissue-specific be a safer way to establish a transplantation protocol in PD. Open-label clinical studies continued through the 1990s have shown that fetal ventral mesencephalic allografts could survive in patients with advanced PD, become functionally integrated, and produce sustained clinical benefits; however, it also soon became clear that transplants of this type produced very variable responses, with some patients showing only little improvement or transient benefits[173]. In patients receiving grafts post mortem studies have demonstrated that also transplanted cells display Lewy bodies, a sign of PD[180, 181].

Overall several issues hinders the further development of a cellular replacement approach for PD[176]. Ethical issues and technical problems (i.e. obtaining fetal and embryonic tissue, immune graft) are slowing down the clinical application in PD patients. New candidate for cell replacement are needed, but the role of other types of potential sources for transplantations - brain-derived adult neural stem cells, adult multipotent stem cells, induced pluripotent cells is still not clear. One case-report describes the effect of autologous transplantation of SVZ derived NSC[140]. Although effects on several clinical aspects were reported, these only lasted 36 months and weaned off after 4-5 years. Based on this result, a phase II study has been approved, but later put on hold due to demands put on cell production facilities (neurogeneration.com).

6.2 Stroke

Stroke is another severe pathology where significant loss of neural tissue is the major factor of the illness. No current therapies promote neuronal recovery following ischemic insults. As mentioned above, endogenous NSC proliferate as a response to both ischemic stroke and subarachnoid hemorrage[182, 183], and stimulation of this endogenous neurogenesis has been tried using a combination with of erythropoietin and hCG as mentioned above.

Based on work in animal models, transplantation of exogenous cells into the injured brain to replace the lost cells or support the remaining cells is one of promising direction[184]. There is a significant experimental background that supports the idea that the grafting of exogenous stem cells from multiple sources can generate neural cells that survive and form synaptic connections after transplantation in the stroke-injured brain[185]. The world's first fully regulated clinical trial of a neural stem cell therapy for disabled stroke patients - PISCES study (Pilot Investigation of Stem Cells in Stroke) – has been started in Scotland at the Institute of Neurological Sciences in 2011. Stem cell therapy (purified population of human neural stem cells, derived from human fetal brain tissue) is being administered to a total of 12 patients. The obtained data is planned to be announced in 2012.

6.3 Spinal cord injury

Cell replacement in spinal cord injury (SCI) is also a field of great interest for neurobiologist and clinicians. A large number of different cells including embryonic and adult stem cells have been transplanted into animal models of spinal cord injury, and in many cases these procedures have resulted in modest sensorimotor benefits[186]. Also a range of clinical experiments involving administration of stem cells for SCI patients have already taken place. Early studies in nine patients showed that unselected human fetal neural tissue transplanted to progressively developing posttraumatic syringomyelia could safely be used to obliterate the syrinx[187, 188]. No tumor developed, but the clinical effect of this obliteration was however limited.

A Portuguese study have reported using unselected olfactory mucosa transplanted into SCI damage site in twenty patients with complete medullary lesions[189, 190]. Treatment resulted in a filling at the transplant site. Urodynamic responses improved in five patients. Two of the patients regained voluntary control of anal sphincter. Eleven patients improved while one patient declined in ASIA impairment scale. The authors concluded that olfactory mucosa autografts are feasible, safe and possibly beneficial.

Geron Corporation (Menlo Park, CA, USA) was in 2009 given a US Food and Drug Administration (FDA) approval for the first test of human embryonic stem cell derived oligodendroglial cells in patients for SCI. Although high controversy existed regarding cell source, safety and patient selection, several patients were included into the study. After an early stop in the study because of worries regarding cyst development at injection sites in preclinical studies, recruitment started in 2010. In the first four patients included in the study, the treatment appeared safe. Sadly, the study was recently stopped due to financial reasons[191].

7. Future directions

Through the last two decades the presence and potential of NSC has become apparent. NSC are used to understand developments of pathology and new based treatments are explored in a range of neurological disease

Although we clearly are at a very early stage of translating the basic biological understanding of NSC into possible therapies, several phase I and II studies have been reported using cell based approaches to treat neurological conditions. However several obstacles affect the translation of promising preclinical studies. Laws, regulation and public understanding of this research are poorly developed. While ethical concerns have develop into regulations that forces restrictive use on a broad range of new technologies in some regions, lack of established safety and quality parameters have led to unsafe and dangerous trials other places[192]. It is a story as old as it is unfortunate, in which opportunistic individuals and companies may manipulate hype and hope for financial gain[193]. Already reports exist on patient developing tumors after ill-designed and unsafe treatment based on NSC[194]. Certainly, at this early stage NSC based therapies should be part of a well designed and publically reported clinical trial (http://www.isscr.org/clinical_trans/pdfs/ISSCRPatientHandbook.pdf).

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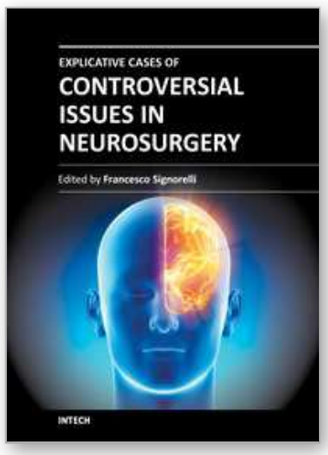

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