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# Neural Stem Cells: Exogenous and Endogenous Promising Therapies for Stroke

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## 1. Introduction

In the last three decades, neuroscience has been profoundly involved in stem cell research that focuses in attempting to develop mechanisms and strategies for secure therapy for different types of brain injury. The limited capacity for cellular regeneration in the highly specialized adult brain makes it particularly vulnerable to cellular damage produced by injuries, such as stroke, which results in a permanent loss of function. Stroke is a a vascular accident characterized by dramatic neuronal loss following a sudden cease of blood flow.

According to the World Health Organization, 15 million people worldwide suffer a stroke event each year. Of those 5 million pass away and another 5 million are permanently disabled (World Health Organization, 2007). Chronic stroke can have a devastating impact on the patient, family, and caregivers; but it also incurs in high economic costs to society. In the United States, stroke is the leading cause of adult disability with an estimated annual medical cost of approximately $40 billion.

High blood pressure contributes to the majority of stroke events, accounting for 12.7 million strokes over the world (World Health Organization, 2007). In developed countries, the incidence of stroke is declining, largely due to efforts in attempting to lower blood pressure and reduce smoking. However, the overall rate of stroke remains high due to the aging of the population.

There are two main types of stroke. One is ischemic stroke, which is caused by a blockage of an artery; the other is hemorrhagic stroke, which is the result of a tear in an artery's wall that produces a blood outflow into the brain. Ischemic stroke is the most common type, it accounts for about 85 percent of all stroke events (Foulkes *et al*., 1988). In both cases, prompt treatment could mean the difference between life and death. Early treatment can also minimize damage to the brain and potential disability. The consequences of stroke on bodily functions and the severity of stroke depend on the affected area of the brain and the extent of the damage.

### 1.1 Current treatments for stroke

In general terms, a stroke event can be divided in acute, sub-acute and chronic phase, by taking in consideration the time course of the injury. The therapeutic strategies to each relative time point are different and centered to ameliorate different sorts of damage.

In the acute phase, the use of thrombolytic agents may dissolve the blood clot in order to slow down or prevent the cascading process that destroys nerve cells after a few hours of ischemic stroke. A currently available and successful intervention to reduce the size of the infarct is the employment of recombinant tissue plasminogen activator (t-PA). T-PA allows the dissolution of a blood clot occluding a cerebral vessel by converting plasminogen into plasmin, an important enzyme present in blood that degrades many blood plasma proteins, most notably, fibrin clots.

Administration of t-PA is approved only within 3 hours of the onset of ischemia, although optimal results are observed if given within 90 minutes (Hacke *et al.,* 2004). Unfortunately, due to this narrow time window as well as a number of contraindications, t-PA therapy is only available to about 5% of stroke patients evaluated in the emergency room. Of these, t-PA may be expected to yield an approximate of 30% increase in the number of patients avoiding long-term neurologic deficits (Ropper & Brown, 2005).

Surgical interventions have enhanced over time and proved to be effective in some cases; however, their success is still below a desired echelon. Although surgical decompression after a stroke event has proved to lessen mortality in severe cases, doubling the probability to survive in a favorable condition, the odds of surviving in a condition requiring assistance from others increases around 10 times (Vahedi *et al.,* 2007). Advances in endovascular techniques may improve recanalization sufficiently to improve patient or cell survival, but these have yet to be substantiated by randomized clinical trials (Burns *et al.,* 2008).

Given the narrow window of time in which thrombolytic drugs and surgical procedures are effective, current research is focused in developing neuroprotective agents that maintain the cellular viability of threatened neuronal tissue (ischemic penumbra) and reduce the secondary damage after ischemic stroke. Many drugs currently undergoing investigation target the excitatory amino acids, such as glycine and glutamate, released by dying neural cells, which are known to lead to downstream changes that destroy nerve cells several hours to several days after a stroke. Dejectedly, although a plethora of neuroprotective compounds have shown promise in animal models, currently their employment has not shown any effectiveness in clinical trials (Dirnagl, 2006).

This fact implicates that nowadays, not a single treatment has been successful in reversing the effects of the chronic stroke. Physical therapy is used to promote functional recovery in long-term stroke patients, but recovery is often incomplete. Therefore, reversal of symptoms after a chronic stroke is a daunting problem that requires the improvement of the patient's lost function achieved by the replacement of lost neurons and glia in the injured region, as well as the establishment of new functional connections. These requirements call for bold new treatments that induce new neural cells to differentiate and integrate into the circuitry that was damaged by the stroke, the cell replacement therapy.

Considering the large amount of data acquired over the past four decades, it has been confirmed that neural stem cells (NSCs) are present throughout life and that thousands of

neurons are born on a daily basis in two specific zones of the brain, the subventricular zone (SVZ) and the hippocampus (For extensive review see Zhao *et al*., 2008).

NSCs are endowed with a self-renewal capacity and are specified to give rise only to nervous tissue-specific cell types, including neurons, glia and oligodendroglia (Reynolds and Weiss, 1996). These features together with the recent finding of the NSCs endogenous response to certain types of insults, such as stroke, lead to the persistent pursue to replace the cellular loss that takes place in the central nervous system (CNS) after injury or neurodegenerative diseases. With the advent of neuroregeneration discipline, new insights have come to the management of stroke. In general, two broad approaches are currently in development for cell replacement therapy in stroke: the recruitment of endogenous neural stem cells and exogenous stem cells transplanted into the affected area.

For a successful therapy, both approaches require to follow a highly regulated process known as neurogenesis, defined as the birth or generation of new neurons from NSCs (Zhao *et al*., 2008). Neurogenesis follows a course where important cellular steps such as proliferation, migration and differentiation (PMD reponse), as well as cell survival are taking place. However, without a doubt, in order to develop a successful cell therapy, more understanding about how the neurogenesis process is occurring is of foremost relevance.

To achieve this goal, some important questions emerge: what are the characteristics required from the microenvironment that allow the neurogenic process to persist throughout life in the adult brain? What are the underlying mechanisms of neurogenesis regulation? And what is the cellular and molecular process regulating neurogenesis under pathological conditions?

In this chapter we will describe the current knowledge about the cellular organization and the molecular regulation that takes place in the SVZ, mechanisms that could provide the basis for the development of cell therapy in stroke and other neurodegenerative diseases. Afterward, the chapter will depict the effects of several growth factors with towering therapeutic potential for their capacity to induce endogenous cell replacement. Finally, this chapter will depict the therapeutic potential of NSCs and other cell types that are suitable for transplantation and ergo, for regeneration therapy in human patients afflicted with cerebral ischemia.

## 2. Cellular and molecular regulation of adult NSCs in the SVZ

### 2.1 The SVZ and rostral migratory stream (RMS): A general view

The study of adult neurogenesis in mammalian CNS began in the 1960's with the pioneering observations made by Joseph Altman, who managed to observe cell proliferation in the adult brain with $^{3}$H-thymidine, a recognized cell division marker (Altman 1963, 1965, 1969). In spite of the controversy, further studies corroborated the existence of brain areas with the potential to generate new neurons from NSCs and defined as neurogenic niches. Nowadays, it is well accepted that the main neurogenic areas in the adult mammalian brain are the SVZ located in the walls of the lateral ventricles and the subgranular layer of the dentate gyrus (DG) of the hippocampus (Figure 1).

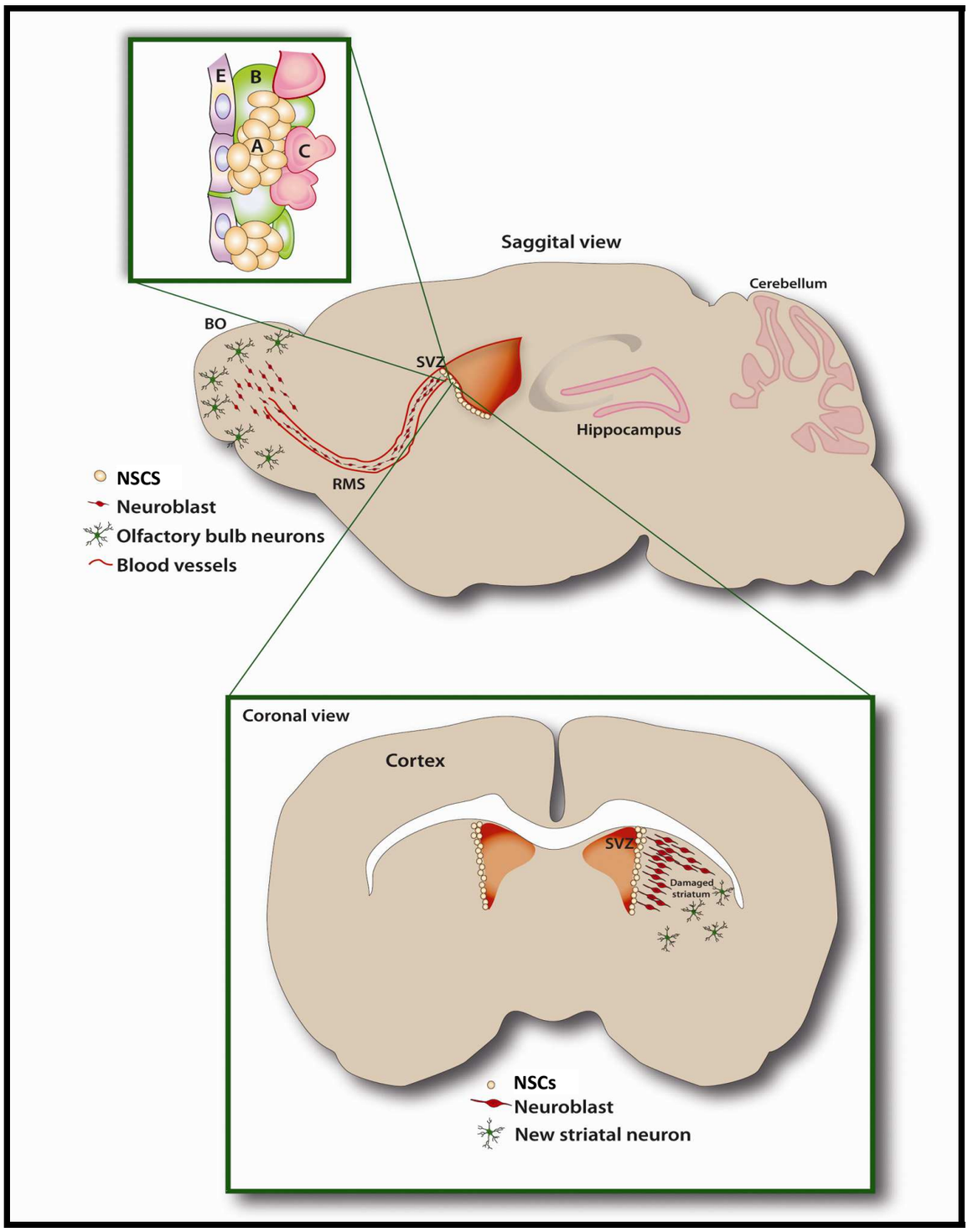


Fig. 1. Stroke and neurogenesis in the subventricular zone .

The SVZ contains many cell types, in addition to chemical and physical factors that create a special microenvironment, conducive to accurately regulate the self-renewal and multipotentiality properties of NSCs (Fuchs *et al*., 2004). It also regulates the neurogenic processes of PMD as well as the less characterized cell integration.

The cellular composition and cytoarchitecture of the SVZ is remarkable peculiar and complex (Figure 1). There are at least five main different cell types integrating the SVZ: Astrocytes, also called B cells, divided in B1 (apical B) and B2 (tangential B); transit amplifying cells (C cells, the putative precursor); neuroblasts (A cells); tanycytes (D cells) and ependymal cells, divided in E1 and E2 (E cells) (Doetsch *et al*., 1997; Mirzadeh *et al*., 2008; Shen *et al*., 2008). To give rise to new neuron, B cells generate C cells and their subsequent division generates A cells that migrate tangentially in clusters via the RMS pathway towards the OB (Figure 1) (Doetsch & Alvarez-Buylla, 1996; Lois *et al*., 1996). In the OB, neuroblasts migrate radially to the granular and glomerular layers, where they differentiate as local interneurons and integrate into functional circuits (Belluzzi *et al*., 2003; Carleton *et al*., 2003; Kosaka *et al*., 1995; Whitman *et al*., 2007). Although uncertain, the functional relevance of the cell replacement that occurs in the OB throughout the lifespan of rodents is attributed to the olfactory adjustment to odor changes in the environment.

Without a doubt, the features shared by the many cellular types of the SVZ contributed to the debate about the true identity of NSCs (for review, Chojnacki *et al*., 2009). Mitotic cells (astrocytes or B cells) have been generally considered to be the true NSCs, owing to their self-renew capacity and their ability to indefinitely produce neuronal and glial progeny (García-Verdugo *et al*., 1998; Doetsch *et al*., 1999). Nevertheless, *in vivo* observations suggest that ependymal cells function as NSCs (Johansson *et al*., 1999). Nowadays, astrocytes are the current accepted NSCs in the adult SVZ. Nonetheless, it is well accepted that the combined stimuli of injury and a growth factor (e.g. transforming growth factor alpha,TGFα) induces a PMD response by NSCs in the ependymal layer and in the SVZ (Gleason *et al*., 2008; Guerra *et al*., 2009).

More recently, the presence of NSCs has been determined in the RMS (Gritti *et al*., 2002), which was considered solely as a migratory pathway for neuroblasts that were migrating rostrally from the SVZ to their final destination, the OB (Lledo *et al*., 2008). This cellular movement within the RMS is called "chain migration", a term established by Lois and coworkers in 1996; they showed that neuroblasts migrated in clusters without axonal guidance or radial glia regulation and instead, used a network of astrocytes that form "glial tubes" (Lois *et al*., 1996). This unique type of migration is thought to enable cells to draw on neighboring cells as their scaffold for migration (Murase & Horwitz, 2002).

The close proximity to the striatum, shared by the SVZ and the RMS raised interest as a cellular replacement option for focal stroke occurring at the basal ganglia. Several molecules have been implicated in the highly regulated neurogenesis processes taking place in both areas. Some of them play multiple essential roles by regulating different levels of the PMD response. For the development of a thriving cell therapy, it is essential to control the processes of proliferation and migration, by means of increasing the number of proliferating cells and redirecting them to the injured area. Therefore, the mechanisms that will allow us to control neuronal fate are of great interest in the neurorepair field and represent a promising subject for the development of a factual clinical treatment.

In the following fraction, we describe the main molecules and cellular events that have been involved in the NSCs differentiation route. Table 1 summarizes the main findings.

### 2.2 Regulation of the NSCs development in the OB pathway

The tangential homotypic traverse of the cells that depart from the SVZ through the RMS is finely controlled by several molecules at different levels. Growth factors are the main mitogenic signals received by NSCs that trigger cell division. A subsequent generation of neuroblasts takes place after such signals; these neuroblasts are confined within the RMS via the combined effect of diffusible chemoattractants and chemorepellents that are flowing at a concentration gradient from the SVZ to the RMS. Noteworthy, the precise migration route is determined by cell-cell interaction mechanisms to ascertain their oriented organization into a continuous alignment. This migration configuration requires individual morphology arrangements in the wanderer neuroblasts, in order to make them suitable for migration.

#### 2.2.1 Growth factors: Regulating multiple effects

Growth factors and neurotrophins have been implicated in the regulation of neurogenesis at early and postnatal development by controlling proliferation, migration and differentiation. Here, we describe some of these factors, including epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), TGFα, brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) for their critical role in these processes.

##### 2.2.1.1 EGF, TGFα and FGF-2: Mitogenic signals

EGF and TGFα, two members of the EGF family, were the first growth factors to be associated to regulate SVZ proliferation through its binding to EGF receptor (EGFR). This tyrosine kynase receptor has been immunodetected in C cells (Doetsch *et al*., 2002) and in astrocytes of the SVZ (Höglinger *et al*., 2004). *In vitro,* studies have demonstrated that NSCs derived from the SVZ proliferate in the presence of EGF or FGF-2, as free-floating neuroespheres that have the potential to differentiate into neurons and glia under appropriate conditions (Doetsch *et al*., 1999; Gritti *et al*., 1999; Reynolds & Weiss, 1992). *In vivo*, intraventricular infusion of EGF and TFGα resulted in an increase of NSCs proliferation in the SVZ (Craig *et al*., 1996; Khun *et al*., 1997; Morshead *et al*., 2003). Grippingly, only TGFα-knockout mice presented a significant reduction in proliferation and neuroblast migration in the SVZ and RMS (Tropepe *et al*., 1997), pointing out its direct role in these two cellular processes.

Alternatively, FGF is a large family that is widely expressed in the central nervous system. Twenty-two members of the FGF family have been identified in humans (Itoh & Orniz, 2011) and have been related to several neuronal processes, for instance, development, adult neurogenesis and repair mechanisms (Reuss & von Bohlen, 2003). To accomplish its mentioned functions, FGF binds with different affinity to each one of the FGF receptor (FGFR) family, integrated by four different members (FGFR 1 to 4) (Ornitz *et al.,* 1996, Johnson & Williams, 1993).

From all the integrants of the FGF family, FGF-2 is the one commonly related to regulate adult neurogenesis. FGF-2 and its receptor, FGFR-1, have been described in the SVZ (Mudó *et al*.,

2007). *In vitro*, FGF-2 stimulates the proliferation of NSCs located in the SVZ and that these progenitors expressing nestin (neural progenitor marker) are able to differentiate into several brain cell types including neurons (Gritti *el al*., 1996, 1999). *In vivo* studies demonstrated that a subcutaneous injection of FGF-2 increases mitotic activity in the adult SVZ (Wagner *et al.,* 1999) and restores the proliferative rates in aging rats (Fluxe *et al*., 2008; Jin *et al*., 2003). Nevertheless, the expression levels of FGF-2 and FGFR-1 in the SVZ do not change during aging, suggesting that although FGF-2/FGFR-1 interaction is involved in cell proliferation, it does not regulate the age-dependent declining of NSCs proliferation (Frinchi *et al*., 2010).

#### 2.2.1.2 BDNF: A multitask molecule

A neurotrophin recognized to undertake multiple roles in SVZ neurogenesis is BDNF. BDNF and its receptors of high and low affinity, TrkB and $p75^{NTR}$, respectively, are expressed all over the SVZ-RMS in a mutually exclusive pattern; with higher expression of BDNF and TrkB in the RMS when compared to the SVZ (Bath *et al*., 2008; Chiaramello *et al*., 2007; Galvão *et al*., 2008; Gascon *et al*., 2007; Giuliani *et al*., 2004; Maisonpierre *et al*., 1990; Snapyan *et al*., 2009; Young *et al*., 2007). In the adult RMS, BDNF mRNA expression was identified in endothelial cells of blood vessels, and absent in astrocytes as well as neuroblasts (Snapyan *et al*. 2009), whereas TrkB was found predominantly in astrocytes (Snapyan *et al*. 2009). Conversely, active TrkB (phosphorylated form) as well as the low affinity receptor $p75^{NTR}$ have been observed in migrating neuroblasts (Bath *et al*., 2008, Galvão *et al.,* 2008; Snapyan *et al*., 2009). $P75^{NTR}$ is also present in a small subset of GFAP-immunopositive cells and transit amplifying cells (C-cells) in the RMS (Bath *et al*., 2008, Galvão *et al.,* 2008; Snapyan *et al*., 2009). The BDNF-TrkB-p75 interaction is correlated to multiple cellular processes in the SVZ-RMS, in particular, those regarding survival, proliferation, migration and cell differentiation.

Luskin and Goldman's groups, who were working independently in a parallel fashion, were the firsts studying the effect of BDNF on adult neurogenesis. They both demonstrated that infusion or overexpression of BDNF into the lateral ventricles increased cell proliferation in the SVZ and migration through the default migratory pathway towards the OB (Benraiss *et al*., 2001; Pencea *et al*., 2001; Zigova *et al*., 1998). Of equal relevance, was their discovery that a higher number of newborn neurons occur in the OB, highlighting its role in differentiation. Simultaneously, new heterotopic spiny neurons were found in the striatum; this finding raises the possibility to redirect the migration of neuroblasts after BDNF infusion, an astonishing breakthrough for endogenous replacement therapy (Benraiss *et al*., 2001; Pencea *et al*., 2001).

More recently, an additional function has been attributed to BDNF, the chemoattraction; defined as the movement of cells towards a chemical concentration gradient. Chiaramello *et al*., tested the hypothesis that the lower expression pattern of BDNF in the RMS and a higher expression pattern in the OB is consistent with the chemoattraction property.

In explants cell cultures, the addition of this neurotrophin resulted essential for survival in addition to, a dose-dependent increase of migrating newly dividing cells. Interestingly, BDNF-induced motility on SVZ neuroblast explants was suppressed by blocking TrkB receptor autophosphorylation and by blocking BDNF action with neutralizing antibodies (Chiaramello *et al*., 2007). Together, these results prove that BDNF has a chemoattractive function through an autocrine and/or paracrine signaling.

Despite the conclusive data concerning BDNF, the mechanisms responsible for maintaining the cells in proper formation while navigating towards the OB core still are unclear. New relevant information comes out from an interesting study from Snapyan and coworkers. They identified BDNF as a molecular signal released from endothelial cells of blood vessels (not from astrocytes or neuroblasts) in the SVZ-RMS, and demonstrated that it promotes neuronal migration via $p57^{NTR}$ activation on neuroblasts (Snapyan *et al*., 2009). They proposed a vasculature-guided migration model in which migrating neuroblasts in the RMS are retained and migrate along this pathway, secondary to the presence of blood vessels that are oriented in a parallel fashion to the RMS (Snapyan *et al*., 2009). This newly proposed model however, does not exclude the previously stated chemoatraction model, since the possibility that both mechanisms are operating is plausible (Snapyan *et al*., 2009).

#### 2.2.1.3 VEGF: Regulator of proliferation and a probable chemoattractive molecule in the SVZ

Another growth factor regulating cellular proliferation in the SVZ is VEGF, a glycoprotein known to be involved in angiogenesis and vasculogenesis. Intraventricular infusion of VEGF leads to an increase in cell proliferation of the SVZ (Jin *et al*., 2002; Sun *et al*., 2006). This finding has been confirmed by inducing the overexpression of VEGF in an ischemic model, where an increase in SVZ proliferation and migration of new cells into the ischemic injury was observed (Wang *et al*., 2007a, 2007b).

The VEGF family ranges from VEGF-A to VEGF-D factors, which have an affinity to several tyrosine kinase receptors: VEGF receptor (VEGFR) 1 (Flt1), VEGFR2 (KDR/Flk1), VEGFR3 (Flt4) and neuropilin receptors (NP1/2) (Matsumoto & Claesson-Welsh, 2001). One of these receptors, VGFR2, has been demonstrated to mediate a chemotactic activity for VEGF in cell cultures (Zhang *et al*., 2003). Its role as a chemoattractive guidance molecule for migrating neural progenitors arising from the SVZ only takes place when these cells are maintained under FGF-2 administration. These *in vitro* essays show that FGF-2 stimulates neural progenitors to express VGFR2, which confers them the capacity to respond to VEGF (Zhang *et al*., 2003).

The chemoattractant function of VEGF in adult brain is still not clear. *In vivo,* intraventricular VEGF administration revealed that VEGFR2/Flk-1 receptors predominate in neurogenic niches such as the SVZ and co-localize with migrating neuroblasts as well as astroglial, endothelial and neuronal cells (Jin *et al*., 2002; Schänzer *et al*., 2004). VEGF expression is observed in astrocytes, being higher in astrocytes of the SVZ and RMS than in astrocytes from non-permissive regions (Balenci *et al*., 2007). This expression represents an endogenous source of VEGF that can be related to neurogenesis and might have a direct effect on the migration of neural progenitors within neurogenic regions (Balenci *et al*., 2007).

### 2.2.2 Chain migration: Molecules implicated

Neuroblast chain formation requires a myriad of specific and regulated interactions to constrain the migrating cells into a precisely organized shape of the RMS. Here, we are describing the main molecules orchestrating the cellular processes involved in chain formation.

#### 2.2.2.1 Ephrin family: Driving proliferation and proper cell positioning in migrating chains

Ephrins are transmembrane-associated proteins that exert their actions through its binding to Ephrin (Eph) receptors; the largest family of tyrosine kynase receptors. Ligands and receptors are divided in two subclasses (A and B), based on their binding properties and structural homologies (Mosch *et al*., 2010). In general, ephrin-A ligands (ephrin-A1 to A6) bind preferentially to EphA receptor (EphA1 to A9), whereas ephrin-B (ephrin-B1 to B3) ligands bind to EphB receptor (EphB1 to B6). The interaction triggers a bidirectional cascade signal, where ephrins mediate a "reverse" signal and receptors mediate a "forward" signal (Cowan & Henkemeyer, 2002).

Ephrins-Eph interaction during development has been implicated in multiple roles, including axonal growth and cell guidance (For review see Wilkinson, 2001). In the adult brain, this interaction has also been related to several roles during neurogenesis (Conover *et al*., 2000; Holmberg *et al*., 2005; Theus *et al*., 2010).

The SVZ and RMS express Eph-B1-3 and EphA4 receptors (Conover *et al*., 2000; Theus *et al*., 2010). In the RMS, EphB2 receptor seems to be surrounding chains of migrating neuroblasts, whereas cells expressing EphB2 in the SVZ are yet still to be determined (astrocytes, neuroblasts or ependymal cells) (Conover *et al*., 2000). On the other hand, ephrin-B ligands are expressed in astrocytes that envelop chains of migrating neuroblast along the RMS pathway. This ephrin/Eph complementary expression pattern seems to regulate the classic ephrin contact-mediated repulsive response to give a position to the cells in specific sites.

Interestingly, blocking Ephrin-B/EphB interactions originates an increase in astrocyte proliferation in the SVZ and promotes a disorganization of the chain network (Conover *et al*., 2000). Therefore, ephrin-B/EphB interaction is a negative regulator of cell proliferation and controls spatial organization in the SVZ. Additionally, this result suggests that inhibition of cell proliferation is required to maintain the germinative niches homeostasis.

Recently, Theus and collaborators revealed the anti-proliferative effect of EphB3, another EphB receptor in the SVZ. EphB3 is expressed in neuronal stem progenitor cells and in neuroblasts. In the traumatic brain injury (TBI) model, where an increase in neurogenesis in the SVZ is induced, Theus's group observed a significant reduction of EphB3 expression, coincidently with enhanced NSCs precursors survival and proliferation post-injury. These findings were corroborated in both ephrin-B3 and EphB3 knockout mice. The two models showed a significant augmentation in SVZ proliferation. Interestingly, in ephrinB3$^{-/-}$ mice, cell division can be reverted by infusion of a soluble form of ephrinB3 (ephrinB3-Fc) in the lateral ventricle. Furthermore, its infusion also prevents TBI-induced neural stem progenitor cell proliferation (Theus *et al*., 2010).

Studies made in ephrin subclass A receptors gave similar results to the ones observed on ephrin-B. The use of ephrin-A2 and ephA7 knockout mice showed that this ligand-receptor interaction is a key inhibitor of cell proliferation in adult brains (Holmberg *et al*., 2005). Ephrin-A2$^{-/-}$ and EphA7$^{-/-}$ mice show an increase in SVZ proliferation concomitant to an increase in the number of new cells in the OB; suggesting that these cells migrate in a regular fashion to the OB. In this study, it was observed that Ephrin-A2 is expressed in neural progenitor cells and neuroblasts of the lateral ventricles, whereas EphA7 receptor

was expressed in ependymal cells and astrocytes of the SVZ. Interestingly, once again it seems that they are localized in a mutually exclusive manner, proper to promote ligand-receptor interactions (Holmberg *et al*., 2005).

Altogether, these findings indicate that ephrin signaling is involved in the regulation of at least two processes of adult neurogenesis, the proliferation and neuroblast guidance into migrating chains, although, the mechanisms controlling these events still have to be determined.

#### 2.2.2.2 ErbB receptors and neuregulins: Chemoattractive implications in proliferation and migration

The ErbB family, also called EGFR family, is integrated by four related tyrosine kinase receptors (ErbB1-ErbB4); one of them involved in cell migration signaling. Anton and coworkers determined that ErbB4 is expressed in neuronal precursor cells (type A cells) residing in the SVZ and the RMS. Additionally, in a small subset of type B cells and type C cells (Anton *et al*., 2004). Ghashghaei and collaborators detected the presence of ErbB4 in a subgroup of CD24+ ependymal cells (Ghashghaei *et al*., 2006).

Experiments in mice lacking ErbB4 receptor, determined its role in the organization of the SVZ-RMS pathway (Anton *et al*., 2004). ErbB4-null mice had a disorganized structure of the SVZ, whereas the neuronal precursors, typically organized in clusters of chains along the SVZ and RMS, were instead forming fragmented chains that migrated as individual cells with an altered orientation. Moreover, impaired placement of interneurons in the OB was observed. *In vitro*, explants from these mutant mice were also unable to form compact neuronal chains, suggesting that loss of ErbB4 disrupts the characteristic "glial" tubular organization in the RMS (Anton *et al*., 2004).

In the SVZ, ErbB4 receptors can be activated by neuroregulins (NRGs), proteins belonging to the EGF family and directly involved in the migration process as chemoattractants (Anton *et al*., 2004). NRGs are a family of four signaling proteins that mediate cell-cell interactions in different organs, including the brain. They have been related to the activation of intracellular signaling pathways that lead to specific cellular responses, including stimulation or inhibition of proliferation, apoptosis, migration, differentiation and adhesion (Yarden *et al*., 2001).

Two NRG types, NRG1 and NRG2, are controlling adult neurogenesis, in particular, cell proliferation and migration. NRG1 type III isoform is highly expressed in the RMS and the OB in the early postnatal development period (Anton *et al*., 2004). *In vitro*, NRG1 type III has been characterized as the preferred chemoattractive protein (compared to NRG1 type I), aiding the migration of neuronal precursor cells from the SVZ (Anton *et al*., 2004).

NRG2 is expressed by immature neuroblasts and in a subset of astrocytes that are lining the ventricles of the SVZ (Ghashghaei *et al*., 2006). Ghashghaei and collaborators demonstrated that NRG1 and NRG2 have different functions in the same niche. Intraventricular infusion of NRG1 induces the aggregation of proliferating precursors into clusters in the SVZ. This aggregation is fundamental for their proper migration, probably by a chemoattractive property. On the other hand, intraventricular infusion of NRG2 promotes astrocyte proliferation and a subsequent increase of neuroblast and GABAergic interneurons in the olfactory bulb (Ghashghaei *et al*., 2006).

#### 2.2.2.3 βI integrins: Receptors regulating chain formation and migration through laminin binding

Integrins are heterodimeric cell surface glycoproteins that regulate proliferation and cell adhesion. Their mechanism of action is mediated through the binding to ECM proteins (fibronectin, laminin), other proteins such as ADAMs (a desintegrin and metalloprotease) and Ig-superfamily cell surface counter-receptors (such as VCAM-1). Two different subunits have been identified, α (18) and β (8), which assemble at least into 24 distinct types of integrins (Hynes, 2002).

Integrins play an important role directing the migration of neuronal precursors demonstrated in both *in vivo* and *in vitro* experiments. At least 11 integrin subunits have been identified in the SVZ-RMS pathway (α1, α2, α3, α6, α7, αv, β1, β3, β5, β6 and β8) with modifications in the temporal expression pattern during development (Belvindrah *et al*., 2007; Murase & Horwitz, 2002). The expression of α1, α6, and α7 integrin subunits in neuroblast of the RMS seems to be higher than the one observed in the SVZ (Belvindrah *et al*., 2007) and grippingly, all of them are able to heterodimerize with a β1 subunit (Hynes, 2002). A model for NSCs-vascular niche adhesion in the SVZ proposes integrins as a receptor for laminin, a protein deposited between neuroblasts and mainly expressed by endothelial cells that surround the SVZ. Laminin is a chemoattractant molecule for SVZ-RMS neuronal precursors that has been proposed to promote cell-cell interactions for chain formation via binding to β1-integrin (Belvindrah *et al*., 2007). As a counterpart, β5 integrin is dispensable for chain migration.

Another integrin, α6β1, is expressed in chain migrating cells along the rodent SVZ-OB pathway, mainly in cell-cell junctions (Belvindrah *et al*., 2007; Emsley & Hagg, 2003; Jacques *et al*., 1998). Laminin is the only known ligand for α6β1 integrin. It has been observed that laminin is recruited to the cell surface of migrating neuroblasts, where it induces chain formation in SVZ explants and the aggregation of neuronal precursors *in vivo*. In addition, it also induces chain migration *in vitro* and *in vivo* (Belvindrah *et al*., 2007; Emsley & Hagg, 2003). Worthy of notice, laminin infusion, in regions where precursor cells are not normally seen, redirects neural precursors toward these locations, as it has been observed in the neostriatum (Emsley& Hagg, 2003). The evidence, strongly supports the idea that laminin-α6β1 interaction is involved in the directional guidance of neuronal precursors, and promotes the formation of cell chains in the adult RMS, as well as maintaining the integrity of glial tubes in the RMS (Belvindrah *et al*., 2007; Emsley & Hagg, 2003).

#### 2.2.2.4 Poly-sialated neural-cell adhesion (PSA-NCAM) molecule: Forming the chain migration process and controlling differentiation

NCAM is a cell surface glycoprotein, member of the immunoglobulin superfamily that mediates cell-cell homotypic interactions, while PSA is a polymer of neuroaminic acid residues added to the NCAM molecule that is highly expressed during development and persists in the adult nervous system. Interestingly, in the adult brain, its expression is particularly confined to neurogenic niches (Seki & Arai, 1993). Specifically, neuroblasts conforming the chain migration in the RMS express PSA-NCAM (Rousselot *et al*., 1995).

In explants cultures, it has been observed, the absence of PSA produced by endoneuraminidase-N (endoN) treatment incites the dispersion of migrating chains, although neuroblasts are still able to migrate as single cells (Hu, 2000). Furthermore, *in vivo* essays demonstrate that these cells do not easily disperse, probably because the tubular glial structures that are ensheating the neuroblasts are confining the migrating cells (Hu, 2000). This clearly indicated that lack of PSA causes a decrease in cell-cell interactions in neural progenitor chains. Therefore, PSA is an important element for neuroblast chain formation (Hu, 2000).

Other studies have demonstrated that PSA is additionally controlling neuroblast differentiation (Battista *et al*., 2010; Petridis *et al*., 2004). Removal of PSA moieties secondary to endoN administration inhibits cell contact dependent differentiation *in vivo*. Furthermore, it promotes a minimal and ectopic dopaminergic differentiation of neural progenitors in the SVZ (Petridis *et al*., 2004). PSA elimination can also result in a dispersal of progenitor cells from the SVZ to the surrounding regions such as cortex and striatum, where neuroblasts differentiate into a calretinin and GABAergic phenotypes (Battista *et al*., 2010).

The effects of NCAM mutations are quite different compared to the ones observed after PSA depletion. NCAM mutations produce a dramatic size reduction of the OB (~30%), while disruption of PSA increases this effect (Cremer *et al*., 1994). PSA-NCAM deficient mice have an altered RMS, where the normal borders are exceeded as a result of an altered accumulation of migrating cells (Chazal *et al.,* 2000).

Altogether, it seems that PSA-NCAM mutations lead neural precursors to fail in the tangential migration to the OB. On the other hand, the ability to migrate radially into the same structure remains unaltered under PSA removal. This was demonstrated after transplanting SVZ cells in the OB of normal and PSA depleted mice OBs (by endoN), in both cases, no differences in radial migration distance or cell morphology were detected, which indicate that PSA does not regulate radial migration (Hu, 1996).

**2.2.2.5 Doublecortin (DCX): Cytoskeletal dynamics for cell translocation**

DCX is a neuron-specific phosphoprotein associated with microtubules that is localized in cell bodies and leading processes. It is involved in the regulation of cytoskeletal dynamics. It is expressed in neuroblasts (type A cells) and immature neurons wherein induces polymerization, in addition to promote the stabilization of microtubules that contribute to cell locomotion (Francis *et al.,* 1999, Gleeson *et al.,* 1999).

DCX expression is downregulated in postmigratory neurons in the OB. Its co-distribution with PSA-NCAM characterizes early committed neurons within the RMS; therefore, DCX is selectively expressed in migratory cell populations within the SVZ, RMS and proximal OB (Moores *et al.,* 2004; Ocbina *et al.,* 2006).

This factor promotes and maintains a bipolar cell morphology, which allows nuclear translocation and therefore cellular migration. DCX deletion results in alterations of the RMS, instigating a thickening of the RMS by the double of its size; it also results in a multipolar neuroblast morphology that correlates to a pause in migration. Furthermore, the migratory cells suffer from an unpaired nuclear translocation towards the centrosome and

undergo some defects in the length of leading processes, which suggests a failure in neurite stabilization (Koizumi *et al.,* 2006).

#### 2.2.2.6 A Desintegrin And Metalloproteinase (ADAM) protein: Propelling migration

ADAM proteins are transmembrane proteins formed by metalloprotease and desintegrin domains. These molecules appear to be related to neuroblast migration, given the fact that other proteins involved in this process require cleavage-dependent activation promoted by ADAMs. The possible mechanism of regulation might be the high capacity of ADAMs to bind to integrins, which, as previously mentioned, are fundamental to this process (Yang *et al.,* 2006). ADAMs are widely expressed in the CNS and approximately 17 different ADAMs have been detected in the CNS, however, so far, only ADAM2 and ADAM21 are directly related to SVZ-RMS cell dynamics (Murase *et al.,* 2008; Yang *et al.,* 2005).

ADAM2 is a protein expressed in the RMS neuroblasts. Lack of ADAM2 results in defects in migration and morphological alterations of the RMS, which is caudally thicker and rostrally thinner, as seen in ADAM2-null mice. ADAM2 probably regulates migration by promoting polarized cell morphology that enables migration, since ADAM2-knockout mice present short leading cell processes and a slow cell migration rate compared with neuroblasts in wild type mice (Murase *et al.,* 2008).

ADAM21 is a protein expressed in ependymal and SVZ cells. The cell processes extending from the SVZ that express ADAM21 are surrounded by glial cells and project to blood vessels that course along the RMS. They are associated with integrin α6β1 in neuroblasts and its location among neural progenitors and neuroblasts suggest that ADAM21 is involved in both cell proliferation and migration (Yang *et al.,* 2005).

#### 2.2.2.7 Chondroitin Sulphate Proteoglycans (CSPGs): Confinement of neuroblast migration

Chondroitin sulfate is a sulfated glycosaminoglycan (GAG), composed of repeated disaccharide units of glucoronic acid (GlcA) and N-acetylgalactosamine (GalNAc). It is commonly attached to proteins as part of a proteoglycan and is a major component of the ECM that interacts with other proteins due to its negative charges (Viapiano & Matthews, 2006).

The adult brain is composed of a type of glia hallmarked by the expression of chondroitin sulfate proteoglycan NG2; which are called NG2 cells. These glial cells are widely distributed in the CNS, are present in multiple branched processes and have the capacity to differentiate into oligodendrocytes (Dawson *et al*., 2003).

NG2 cells are aligned along the border of the SVZ and are more abundant as they are further distal to the RMS, being higher in the OB. This spatial pattern suggests a correlation between neuroblast maturity and the presence of NG2 cells. Furthermore, in the glomerular cell layer of the OB, these cells are in direct contact to cells expressing immature and mature neuronal markers (DCX, PSA-NCAM and the neuronal nuclear antigen, NeuN). The spatial organization of NG2 in SVZ-RMS raises the possibility that these cells confine the migration of neuroblasts within the RMS and prevents its dispersion (Komitova *et al*., 2009) However, to make this assumption further research is required.

#### 2.2.2.8 Slit and Robo proteins, chemorepulsive interactions for appropriate migration

Slit and Roundabout (Robo), ligand and receptor respectively, are evolutionarily conserved proteins in *Drosophila* and vertebrates. In mammals, Slit1-3 and Robo1-3 have been identified and implicated in axonal repulsion and cell guidance (Brose and Tessier-Lavigne 2000). Slit is a secreted protein that binds directly to Robo and functions as a chemorepellent for OB axons (Li *et al*., 1999).

Slit and Robo function on the migratory pathway just started to be unraveled through *in vitro* experiments. Brain explants show that Slit 1 and Slit2 are expressed in the septum, where it repels progenitor cells rising from the SVZ and maintained along the RMS (Wu *et al*., 1999). Moreover, Slit2 is expressed in the choroid plexus where it repels neural progenitors (Hu, 1999). Given the fact that Robo2 and Robo3 receptor expression has been determined in the SVZ and RMS (Marillat *et al*., 2002), it is presumable that a Slit-Robo interaction is occurring in the migratory stream.

Slit1 deficient mice contributed to clarify the role of Slit1 in SVZ migration. Neuroblasts raised from the SVZ of knockout Slit1 mice migrate caudally to the corpus callosum, rather than to the RMS, which supports the idea that Slit1 plays an important role in directing migration. Moreover, Slit1 is also expressed in type A and type C cells within the SVZ and RMS, indicating that Slit presence is not limited to chemorepulsion activity, but possibly in parallel way, act as a individual cell migration inhibitor and might maintain chain migration integrity because Slit-mutant neurospheres migrate farther and in a disperse manner (Nguyen-Ba-Charvet *et al*., 2004). Therefore, the evidence suggests that Slit1 and Slit2 are involved at least, in the beginning of the cell migration pathway from the SVZ towards the OB, orchestrating migration through a concentration gradient (Wu *et al*., 1999).

#### 2.2.2.9 Semaphorin-Neuropilin complex: Does it regulate SVZ-RMS migration?

Semaphorins are axonal guidance molecules with attractant or repellent activity that participate in early development, angiogenesis and cell migration (Tamagone & Comoglio, 2000). In vertebrates, semaphorins are divided in two groups; class 3 for secreted semaphorins and classes 4 to 7, which include transmembrane semaphorins (Raper, 2000). There is one receptor family manly involved in the regulation of semaphorin responses in the CNS: The neuropilins, NP1 and NP2 (De Wit & Verhaagen, 2003; Raper, 2000). Neuropilins are preserved throughout the entire adulthood (Giger *et al*., 1998) and it is well known that these receptors maintain and stabilize neuronal connections and prevent axonal sprouting (Giger *et al*., 2000; Wit & Verhaagen 2003).

Semaphorin 3A and its homodimer receptor NP1 are present along the entire RMS in the adult brain; they appear to be related to the regulation of neuroblast migration. NP1 is located in endothelial cells and binds to VEGF, an important angiogenic factor (Soker *et al*., 1998). Therefore, it has been suggested that semaphorin 3A modulates angiogenesis. This might support the notion that the guidance of migrant neuroblasts chains could be regulated by semaphorin 3A through the indirect action of remodeling blood vessels (Melendez-Herrera *et al*., 2008).

| Complex/Molecule | | Migration /Participate in proliferation(*) | Cell type expression | | Reference |
|---|---|---|---|---|---|
| | | | Receptors | Ligands | |
| Growth Factors | **EGF-EGFR** | Negative effect on neuroblast migration (*+) | EGFR:C cells, neuroblasts, and astrocytes | Neuronal precursor cells | Craig *et al*., 1996; Doetsch *et al*., 1999; Doetsch *et al*., 2002; Gritti *et al*., 1999; Kim *et al*. 2009; Morshead *et al*., 2003; Reynolds & Weiss, 1992; Tropepe *et al*., 1997 |
| | **EGF-TGFα** | Neuroblast migration (*+) | | TGFα:Astrocytes | |
| | **BDNF-TrkB** | Autophosphorylation induces motility on SVZ neuroblasts (*+) | TrkB:migrating neuroblasts TrkB-T1: astrocytes and ependymal cells | BDNF:Endothelial cells | Maisonpierre *et a*l., 1990; Giuliani *et al*., 2004; Chiaramello *et al*., 2007; Gascon *et al*., 2007; Young *et al*., 2007; Galvão *et al*., 2008; Snapyan *et al*., 2009 |
| | **BDNF-P75NTR** | Promotes neuroblast migration | P75NTR: Neuroblasts, astrocytes and C-cells | | |
| | **FGF-2** | Cell guidance with VEGF interaction (*+) | FGFR: Neural precursors (Nestin+) | FGF-2:Glial cells | Mudó *et al*., 2007; Frinchi *et al*., 2010 |
| | **VEGFR-VEGF** | Neuroblasts guidance (*+) | VEGFR: Neuroblasts | VEGF:Astrocytes, endothelial cells | Jin *et al*., 2002; Zhang *et al*., 2003; Schänzer *et al*., 2004; Schmidt *el al*., 2009 |
| Tyrosine kinase receptors | **Ephrins** | Chain organization (*-) | EphB3:C cells and neuroblasts EphA7:Ependymal cells and astrocytes | Ephrin B:Astrocytes Ephrin A2:Neuroblasts | Conover *et a*l., 2000; Holmberg *et al*., 2005 |
| | **Erb** | ErbB4:Chain organization NRG1:Neuroblast aggregation NRG2:SVZ-cell organization (*+) | ErbB4:Neuroblasts, C cells, ependymal cells | *NRG1*:Neuroblasts *NRG2*:Neuroblasts and astrocytes | Anton *et al*., 2004; Ghashghaei *et al*., 2006 |
| **Integrins** | **Integrins** | Neuroblast aggregation and chain formation (*+) | α6β1-integrin: Neuroblasts and NSCs | Laminin:Recruited to the cell surface of neuroblasts Highly abundant around blood vessels in endothelial cells | Jacques *et al*., 1998; Emsley & Hagg, 2003; Belvindrah *et al*., 2007; Shen *et al*., 2008 |

| Complex/Molecule | | Migration /Participate in proliferation(*) | Cell type expression | | Reference |
|---|---|---|---|---|---|
| | | | Receptors | Ligands | |
| **Immunoglobulin superfamily** | **PSA-NCAM** | Chain formation. Cell adhesion for translocation | - | Neuroblasts and non-migrating glial progenitors | Seki and Arai 1993; Hu *et al.*, 1996; Chazal *et al.*, 2000; Petridis *et al.*, 2004 |
| **Microtubule-associated proteins** | **DCX** | Stabilization of microtubules. Bipolar morphology for nuclear translocation | - | Neuroblasts | Francis *et al.*, 1999; Gleeson *et al.*, 1999; Moores *et al.*, 2004; Ocbina *et al.*, 2006 |
| **Extracelular matrix molecules** | **ADAMs** | Chain formation. Maintains cell morphology | Integrins | ADAM2:Neuroblasts (RMS) ADAM21:Ependymal and SVZ cells | Komitova *et al.*, 2009; Murase *et al.*, 2008 Viapiano *et al.*, 2006; Yang *et al.*, 2006 |
| | **CSPGs** | Neuroblast migration | Integrins | NG2 glial cells | Viapiano *et al.*, 2006; Komitova *et al.*, 2009 |
| | **Tenascin-R** | Radial migration in OB | Not determined | Granular layer of the OB | Saghatelyan *et al.*, 2004 |
| **Slit-Robo** | **Slit-Robo** | Chemorepulsion | Robo1:OB Robo2 and Robo3: SVZ-RMS neuroblasts | Slit1 and Slit2:Septum Slit1:Type A and C cells | Li *et al.*, 1999; Wu *et al.*, 1999; Marillat *et al.*, 2002; Nguyen-Ba-Charvet *et al.*, 2004 |
| **Semaphorin-NP** | **Semaphorin-NP** | Suggested that modulates neuroblast migration | NP1:Endotelial cells (RMS) | Semaphorin 3A: Endothelial cells (RMS) | Tamagone & Comoglio 2000; Meléndez -Herrera *et al.*, 2008 |
| **Reelin/ApoER2-VLDLR** | **Reelin/ ApoER2-VLDLR** | Radial migration in the OB by cell detachment | ApoER2-VLDLR: Neuroblasts | Reelin:Mitral cells of the OB | D´Arcangelo *et al.*, 1999; Hack *et al.*, 2002; Simó *et al.*, 2007 |
| **Prokineticin2** | **PK2/Prokr2** | Cell detachment, radial migration in OB | Prokr2: OB Glomerular layer | PK2: OB Glomerular layer | Ng *et al.*, 2004; Prosser *et al.*, 2007 |

(*+) Increase cell proliferation, (*-) Decrease cell proliferation

Table 1. Regulation of NSCs proliferation and neuroblast migration in the SVZ-RMS pathway.

#### 2.2.2.10 Radial migration in the OB: Reelin, Tenascin-R and Prokineticin2 as detachment signals

After completing chain migration through the RMS, neuroblasts finally arrive to the OB, where a shift in migration occurs, from a tangential to radial direction. To accomplish this cellular step, a neuroblast detachment signal is required. The most described ligand-receptor complex involved in this process is reelin/ApoER2-VLDLR. Reelin is an ECM secreted glycoprotein expressed in mitral cells of the OB. The surface receptors for reelin are the apolipoprotein E receptor 2 (ApoER2) and the very low-density lipoprotein receptor (VLDLR), both expressed in migrating neuroblasts. To allow the migratory switch, binding of reelin to its receptors induces phosphorylation of the intracellular adaptor protein disabled-1 (Dab1) and Src family kinases (SFK) (D´Arcangelo *et al.*, 1999, Hiesberger *et al.*, 1999).

*In vitro*, SVZ explants supplemented with reelin exhibit a loss of neuroblast chain formation, which gives raise to individual cell migration. Furthermore, *in vivo* observations of reeling-null mice reveal that neuroblasts fail to migrate radially in the OB and remain in clusters (Hack *et al.,* 2002). Additionally, the experimental overexpression of reelin by grafting reelin-expressing cells in the SVZ produces a dispersion of neuroblasts around the ventricular structures by chemokinetic activity and detachment (Courtes *et al.,* 2011).

Recently, another function of reelin has been hypothesized. It was demonstrated that reelin expression is reactivated after brain injury (focal demyelinization of corpus callosum) in mature neurons at the proximal damaged area. It is suggested that reelin enhances chemoattraction exerted by lesion-derived cytokines that contribute to neuroblast recruitment in the boundary of the damaged area. All together, these results support reelin's performance as a detachment and migration key factor (Courtes *et al.,* 2011).

There are other molecules involved in radial migration in the OB; one of these molecules is tenascin-R, an ECM glycoprotein expressed in the granular layer of the OB, where it initiates neuroblast chain detachment and radial migration. Tenascin-R-null mutant mice show a cell reduction in the granular layer, whereas neuroblasts remain in clusters in the OB (Saghatelyan *et al*., 2004).

Finally, other molecule involved in OB migration is Prokineticin2 (PK2), which is expressed in the granular and periglomerular layers of the OB and acts as detachment signal and chemoattractant. PK2 receptor-null mice (Prokr2) exhibit a decrease in the volume of the OB and have an abnormal accumulation of neuroblasts around the olfactory ventricle. This suggests a deficiency in neuroblast migration and defects in chain migration detachment (Prosser *et al*., 2007). Furthermore, i*n vitro*, SVZ explants co-cultured with cells obtained from the glomerular layer of the OB, begin cell migration toward the glomerular layer of the explants; whereas cells from the glomerular layer of PK2-null mice do not exhibit chemotactic activity (Ng *et al.,* 2005).

## 3. Growth factors as an endogenous approach for neurorepair therapy

### 3.1 Endogenous response after stroke

We just described in the previous section the existence of a substantial number of molecules and its complex signaling regulating PMD response at the SVZ under basal conditions.

However, studies also reveal that neurogenesis could be triggered secondary to specific injury conditions, including stroke. Focal cerebral ischemia promotes neurogenesis in the DG of the hippocampus and in the SVZ (Arvidsson *et al*., 2002; Jin *et al*., 2001; Liu *et al*., 1998; Parent *et al*., 2002; Zhang *et al*., 2001); both being a feature shared by rodent and the human brain (Jin *et al*., 2006; Martí-Fabregas *et al*., 2010). Moreover, these studies demonstrate that the default migratory pathway, followed by neuron precursors from the SVZ that supply the OB region, can be partially diverted to other destinations including the striatum and cortex after stroke injury. Recently, it was demonstrated that stroke enhances long-term neurogenesis, although, decreased in magnitude when compared to the acute phase (Thored *et al*., 2006). These findings are of paramount relevance for their therapeutic potential in the field of neuronal damage reestablishment, by taking advantage of an endogenous cell source. However, Ardvisson *et al*., in their seminal work, determined that within 6 weeks of transient focal stroke uniquely 0.2% of newborn neurons from the SVZ were integrated in the damaged striatum. Thus, neurogenesis occurring after cerebral ischemia represents an insufficient cell source for the purpose of neuronal replacement therapy (Ardvisson *et al*., 2002).

### 3.2 Self-renewal induced by growth factors: The endogenous neuronal repair

The rationale for self-renewal induced by growth factors relies on the existence of neurogenic niches in the brain with the potential to modulate the proliferative response by either injury or growth factors. The purpose of this approach has been to determine whether growth factors are able to amplify the endogenous response of NSCs in a meaningful level that can account an increase of neuronal differentiation. The concept however, was not new in the field of stroke studies. One of us, Dr. James Fallon (Fallon *et al*., 2000) employed the 6-hydroxydopamine lesion rodent model of Parkinson's disease, where he and collaborators demonstrated that proliferating cells significantly increased in the striatal SVZ ipsilateral to the injured side, however, this increase was exclusive in animals that also received an striatal infusion of TGF-α; neuroblasts then migrated in mass into the striatum to become neurons.

There is a long and increasing list of growth factors that has the potential to be implemented as a therapeutic tool in the recovery process after a stroke event, exploiting their neuroprotection and neurogenesis features. Here, we are focusing on studies that unveil that growth factors are modifying neurogenesis subsequent to the onset of focal cerebral ischemia, specifically in the SVZ-RMS. Table 2 represents a chronological compendium of such studies. The hematopoietic factors known to compel neurogenesis were omitted in our chapter, nonetheless, they are extensively reviewed by others (Greenberg & Jin, 2006).

The majority of the research described in this section of the chapter employs the most common method to study focal stroke, the middle cerebral artery occlusion (MCAO) model, which affects the striatum and/or cortex, resembling the injury that commonly occurs in stroke patients (Figure 1). To induce this kind of ischemia, an incision in the neck is made and after exposition of the common, external and internal carotid arteries, a monofilament suture is then carefully introduced via the external carotid artery through the lumen of the internal carotid artery until it reaches and occludes the middle cerebral artery (MCA).

### 3.2.1 EGF family of growth factors

It is important to highlight that in practically all the protocols regarding neurogenesis, the quintessential strategy to identify newborn cells is the administration of bromodeoxyuridine (BrdU), a marker of cell division, which intercalates into the DNA of cells that are undergoing proliferation. The neuronal (or glial) lineage of BrdU-labeled cells is determined by the co-staining with specific markers of immature or mature phenotypes, which may differs depending on the goal of the study.

#### 3.2.1.1 EGF

Teramoto and collaborators reported in 2003 the first study about the effect of a growth factor on neurogenesis in the SVZ after an ischemic insult (Teramoto *et al*., 2003). The antecedents implicating EGF as a factor that promotes NSCs proliferation and migration from the SVZ to the striatum lead them to test if EGF could induce a PMD response in the damaged brain after an ischemic event. They administered an intraventricular dose of EGF two days after stroke for a period of one week that allow a noteworthy neuronal replacement increase after week 13$^{th}$ post MCAO. Interestingly, Teramoto determined that the BrdU+/NeuN+ cells observed at the boundary zone of the stroke lesion were not DARPP-32 (dopamine and adenosine 3′,5′-monophosphate–regulated phosphoprotein of 32KD) positive, the prevailing neuronal population in the striatum. Rather than, they found 65% of the new cells maturated into aspiny parvalbumin-containing (PV) interneurons. This effect is intriguing, given the fact that *in vitro,* EGF stimulates the DARPP-32 phenotype (Reynolds & Weiss, 1992). Therefore, albeit EGF is probably directing the mechanisms related to neuronal commitment and fate specification, such claims need further study.

#### 3.2.1.2 Heparin-binding epidermal growth factor-like growth factor (HB-EGF)

Another member of this family is the HB-EGF that also has a clear effect in increasing proliferation of NSCs in the SVZ. However, its effect on migration and differentiation is not fully disclosed. In one study, Jin and coworkers analyzed such effect 4 weeks after stroke and observed an increase in the number of BrdU cells (approximately 40%) and the number of BrdU cells co-localizing with the immature neuronal marker TUC-4 in the SVZ. Nonetheless, a concomitant decrease of approximately 60% of neuroblasts (DCX+) migrating towards the striatum was also observed. The authors suggested that this effect is probably due to its chemoattractant properties. In spite of the decrease in migration, a significant improvement in neurological outcome was accounted, probably due to a reduction in infarct size promoted by the growth factor (Jin *et al*., 2004). Later on, another study conducted by Sugiura and coworkers determined that recombinant adenovirus-expressing HB-EGF promotes neurogenesis and angiogenesis in the SVZ. Nonetheless, opposite to the study developed by Jin *et al*., no effect limiting migration of newborn cells toward the striatum was reported. Therefore, also in contrast, an increase of neuronal cells was observed in the ischemic striatum, going from 2 BrdU+/NeuN+ cells to 23 cells per mm$^2$ (Sugiura *et al*., 2005) Additionally, a functional recovery was observed. The discrepancy in both results still remains to be discerned, however, it could be due to the different methods employed to deliver the growth factor, which impose differences in concentration and availability.

#### 3.2.1.3 TGF-α

The first report of TGFα's effect on neurogenesis in rats that were subjected to transient MCAO, was made by our group (Guerra-Crespo *et al*., 2009). The majority of stroke studies analyze the effect of growth factors in the acute phase of ischemia. However, we were interested in the delayed administration of the growth factor, since it resembles the typical situation in humans with preexisting stroke injuries, who might benefit from this type of therapy. Therefore, we infused TGFα directly in the striatum four weeks after injury, when the infarction area was no longer expanding and the cellular deterioration had stabilized, indicating that the acute phase was completed and the chronic phase had begun.

Eight weeks after MCAO, and four weeks after the onset of TGFα administration, we found a 4-fold increase of BrdU labeled cells arising from the ependymal layer and the SVZ. Many of the BrdU-labeled cells of the SVZ and others under migration were expressing the immature neuronal marker Meis2, a transcription factor that is strongly expressed in striatal precursors. Additionally, we found around the site of the infarction about a 7-fold increase of BrdU cells that were co-labeled with the neuronal-fate marker NeuN, whereas, several of the newborn cells co-labeled with DARPP-32, indicating that they differentiate into striatal neurons, which are typical for this brain region. These results indicate that TGFα treatment significantly increased the yield of neurons produced in the injury response. Although we did not examine the long-term survival of these neurons, an approximately 90% of behavioral recovery (corner and cylinder test) in the chronic animal suggests that many of them became functionally and integrated in the host's CNS (Guerra-Crespo *et al*., 2009).

In the same year, another group (Leker *et al*., 2009) reported an increase in neurogenesis as well as angiogenesis induced by TGFα. Leker *et al*., administered an intraparenchymal dose of the growth factor 1 day after ischemia, for a consecutive period of 14 days and analyzed the long-term response. TGFα increased the number of BrdU cells and allow a 2-fold increase of neuroblasts in the ischemic hemisphere. Nonetheless, only a slight number of newborn neurons in proximity to the infarct border were observed, suggesting that under these specific experimental conditions, TGFα leads to a moderate but significant neuronal differentiation. Concomitant to neurogenesis, TGFα expanded (2.4-fold) the area covered by blood vessels in the ischemic border zone. The mechanism involved the recruitment of endothelial bone marrow-derived cells into newly formed cerebral blood vessels.

#### 3.2.1.4 Intranasal infusion of TGFα

The employment of growth factors in patients is impeded by the facts that intracranial infusion is impractical and that many growth factors intravenously administrated are not able to cross the blood-brain barrier (BBB). Even though there are methods for bypassing the BBB, they typically consist of invasive neurosurgeries that would restrict clinical application to the most severe cases. Noninvasive techniques that are capable of delivering growth factors to the CNS represent a therapeutic alternative to surgery. A pioneer work was the intranasal administration of neurotrophic growth factor (NGF), which demonstrated that delivered growth factors could bypass the BBB. Since then, the prevalence of the intranasal administration technique for CNS treatment has grown considerably (Capsoni *et al*., 2002; De Rosa *et al*., 2005; Frey *et al*., 1997; Liu *et al*., 2004; Ma *et al*., 2008). With this approach of administration, therapeutic molecules traverse the BBB through the olfactory pathway and

the less-studied trigeminal neural pathway (Thorne *et al*., 2004). The advantage of this method is that factors are delivered directly into the brain and thereby avoid adverse systemic effects. The simplicity of the intranasal administration of growth factors makes it an outstanding strategy. This non-surgical approach represents a potential therapeutic strategy for human patients.

Based in this knowledge, we delivered an intranasal dose of a pegylated form of TGFα (PEG-TGFα) to make it more stable for the nasal route. We found that intranasal delivery is a viable alternative because PEG-TGFα was able to induce the proliferation and migration of neural progenitors to the damaged striatum (in terms of BrdU incorporation and nestin expression), although of less magnitude compared to intracranial TGFα administration. This finding is associated with significant behavioral improvement in the MCAO model, measured by the corner and cylinder test. Therefore, intranasal delivery of PEG-TGFα holds great therapeutic potential.

### 3.2.2 FGF-2

From the FGF family, FGF-2 is the member which neurogenic properties have been demonstrated. The first study, performed in 2003, reported that, regardless of a 30% increase of BrdU+/DCX+ cells, no BrdU+/NeuN+ cells were observed (Wada *et al*., 2003). In the same year, Matsuoka and collaborators also reported an increase (2.1 fold) of BrdU+ cells in the SVZ of a global ischemia rodent model, through FGF-2 gene delivery by an adenoviral vector. Although neuronal differentiation in the striatum was not analyzed, a small number of newborn cells (3%) were labeled positive for NeuN amongst the different layers of the cortex and by the proximity to the SVZ suggesting that such cells were derived from the SVZ (Matsuoka *et al*., 2003). Matsuoka's research set a precedent on gene therapy field, since was the first time that a growth factor was administered through viral vector in a stroke model

Both former works were focused on the study of acute phase of stroke, where a short infusion period of the growth factor or a short time analysis after the growth factor-adenovirus transduction was made. More recently, Leker and coworkers employed an ischemia model with predominant cortical damage and an adenovirus delivery system that allowed overexpression of FGF-2 for a long time period. They were able to observe that FGF-2 increased proliferation and migration from the SVZ and that the immature neurons were localized in the border of damaged cortex when analyzed at 30 and 90 days after stroke. Additionally, the results shown that the group treated with FGF-2 presented a 2-fold increase of newborn cells expressing NeuN and even a higher increase (22 *versus* 1.6%) of cells labeled with the immature neuronal marker Hu. In either Matsuoka *et al*., or Leker *et al*., studies, no lineage analyses was performed to asseverate without a doubt, that such cells were originated in the SVZ, since they could be also produced in the cortex. Nevertheless, the evidence suggests that the majority of the cells arose from the SVZ (Leker *et al*., 2007).

The previous studies disclose the enhancement achieved on endogenous neurogenesis in the SVZ when EGF or FGF-2 are administered independently. However, a more interesting fact would be to analyze the simultaneous effect of both growth factors in order to increase neurogenesis. Nakatomi *et al*., in a breakthrough work addressed that question in a transient ischemia model with specific pyramidal hippocampal damage. The results demonstrated

that endogenous proliferation and migration are enhanced by intraventricular co-administration of growth factors after stroke. However, that the growth factor induced neuronal regeneration of approximately 40% of the CA1 pyramidal layer, one month after stroke, was a remarkable finding (Nakatomi *et al*., 2002). This study was centered in the analysis of the hippocampus; however, only a few years later, Baldauf and Reymann examined the combined effect of EGF/FGF-2 on the SVZ. They observed an increase (almost double) in the number of BrdU+/DCX+ cells in the ipsilateral striatum, in spite of a concomitant increase in the infarct volume. Unfortunately, neuronal fate and behavior were not analyzed; therefore, further analyses are required to conclusively discern the effect of the synchronic administration of EGF and FGF-2 for striatum neuronal replacement (Baldauf and Reymann 2005).

### 3.2.3 Neurotrophic factors: BDNF

The neuroprotective effects of the intraventricular infusion of BDNF after global ischemia were reported for the first time in 1994 (Beck *et al*., 1994). However, the role of BDNF in neurogenesis was recently demonstrated. In one report (Gustafsson *et al*., 2003), BDNF expression was successfully attained in the substantia nigra by means of an adenovirus; the objective was to transport BDNF anterogradely to the striatum and avoid damage in its cytoarchitecture. The transduction of the viral vector took place 4 to 5 weeks prior to the MCAO. With this strategy, they found a significant increase of neuroblast migration towards the striatum at 2 weeks after ischemia. Additionally, a higher number of BrdU cells co-laballed with early neuronal marker Hu and striatal neuronal marker Meis2 were observed. The significant relevance for replacement therapy cannot be established with these findings, because the transduction of the viral vector generated anomalous behavior patterns and therefore, the behavioral outcome cannot be correctly analyzed. Moreover, the high expression levels of BDNF aggravated the cellular death of cholinergic, PV and neuropeptide Y interneurons in the striatum, which could override the neuroprotective effects observed in former studies performed by the same research group (Andsberg *et al*., 2002).

Some years later, in another attempt to determine the effect of BDNF on neurogenesis, Schavitz and coworkers induced a parietal cortical lesion, and after an intravenous injection of BDNF on the following 5 days, were able to observe precursor cells rising from the SVZ that were migrating toward the ipsilateral striatum. A substantial number of neuroblasts were recruited to the ischemic hemisphere 37 days after the last injection; still, neuronal replacement was not observed with this paradigm of cortical injury, since no BrdU+/NeuN+ cells were observed. Additionally, the authors were unable to detect cortical neurogenesis (Schavitz *et al*., 2007).

In summary, a variety of growth factors have been tested in experimental stroke models and irrespective of their nature, practically all of them can induce proliferation on NSCs that reside in the SVZ. Most of them, enhance in a parallel fashion the recruitment of neuroblasts toward the peri-infarcted area, either striatum or cortex. However, extensive differences in neuronal differentiation have been observed in the damaged area (even when employing the same growth factor), ranging from a highly significant increase to a decrease in differentiation levels. The behavioral outcome, measured as an indirect index of functional

integration, has been analyzed only in the minority of the studies, finding an important level of recovery in some of them.

The potential of growth factors for endogenous cell replacement is evident; however, clinical studies are not currently being undertaken, given the fact that growth factors encompass a strong and dangerous mitogenic effect.

| Growth factor | Stroke Model | Delivery Method | Time period of infusion | Effect on Neurogenesis | Behavioral outcome | Reference |
|---|---|---|---|---|---|---|
| **EGF Family** | | | | | | |
| **EGF** | Mouse Left MCAO | Minipump in lateral ventricle | 21 days after ischemia for 7 days | - 65% of new PV interneurons 13 weeks after stroke<br>- 100 fold of neuronal replacement | NA | Teramoto *et al.*, 2003 |
| **HB-EGF** | Rat Right MCAO | Minipump in lateral ventricle | 24 hours after ischemia for 3 days | - Increase of BrdU+ cells expressing TUC-4<br>-60% decrease in migrating neuroblasts 4 weeks after stroke | Improvement in neurological score | Jin *et al.*, 2004 |
| **HB-EGF** | Rat Left MCAO | Adenoviral vector in lateral ventricle | 3 days after ischemia | - 2-fold increase of vascular density (angiogenesis)<br>- Increase of BrdU+/NeuN+ cells (2 vs 23 cells per $mm^2$) 28 days after ischemia | Improvement in rotarod test | Sugiura *et al.*, 2005 |
| **TGFα** | Rat Left MCAO | Minipump in striatum | 1 month after ischemia for 28 days (chronic treatment) | - 4-fold increase of BrdU+ cells<br>- 7-fold increase of BrdU+/NeuN+ cells and several BrdU+/DARPP-32+ two months after stroke | 90% of improvement in corner and cylinder test | Guerra-Crespo *et al.*, 2009 |
| **TGFα** | Mouse Left MCAO | Minipump intra-parenchyma | 1 day after ischemia for 14 days | - 2.4 fold increase in blood vessel cover area in the infarct border<br>- Small number of newborn neurons in the infarct border 90 days post-infusion | NA | Leker *et al.*, 2009 |
| **TGFα** | Rat Left MCAO | Intranasal delivery | 4 intranasal doses per month (1 per week) | - Increased BrdU+ cells in SVZ and ischemic striatum<br>- Increased neurogenesis (BrdU+/Nestin+ cells) in the peri-infarcted striatum | 50% of improvement in corner and cylinder test | Guerra-Crespo *et al.*, 2010 |

| Growth factor | Stroke Model | Delivery Method | Time period of infusion | Effect on Neurogenesis | Behavioral outcome | Reference |
|---|---|---|---|---|---|---|
| **EGF Family** | | | | | | |
| **FGF-2 (bFGF)** | Rat Right MCAO (cortex) | Intracisternal injection | Injections 24 and 48 hrs after stroke | - 30% increase of BrdU+/DCX+ cells at day 7 and 2% at day 21<br>- No BrdU+/NeuN+ cells | NA | Wada *et al.*, 2003 |
| **FGF-2** | Gerbil Global ischemia | Adenoviral vector in lateral ventricle | Transduction 3 hrs after stroke | - 2.1 fold increase of BrdU+ cells in the SVZ 7 days after ischemia<br>- 3% of BrdU+/NeuN+ in cortex 30 days post-stroke | NA | Matsuoka *et al.*, 2003 |
| **FGF-2** | Hypertensive Rat MCAO (cerebral cortex) | Adenoviral vector in the infarct border | Transduction starting the same day of ischemia (chronic treatment) | - Increase of BrdU+ cells expressing neural transcription factors MASH1 and Pax6 in the peri-infarcted area 30 days after stroke<br>- Increase of BrdU+/Hu+ cells (22% vs 1.6%) | Improvement in motor disability score | Leker *et al.*, 2007 |
| **FGF-2+ EGF** | Rat Left MCAO | Minipump in lateral ventricle | 10 min after ischemia for 14 days | - Increased proliferation in the striatum<br>- 2 fold increase of BrdU+/DCX+ cells in striatum 14 days after stroke | NA | Baldauf *et al.*, 2005 |
| **Neurotrophin** | | | | | | |
| **BDNF** | Rat MCAO | Adenoviral vector in SN transported anterogradely towards striatum | 5 weeks previously to MCAO | - Increased neuroblasts (DCX+)<br>- Increased neuronal death in the striatum<br>- Increased BrdU+/Meis2+ cells 2 weeks post-ischemia | Vector-induced abnormal motor behavior | Gustafsson *et al.*, 2003 |
| **BDNF** | Rat Photothrombotic ischemia (cortical stroke) | Intravenous injection | 1 hour post-ischemia 2nd to 5th day | - Highly significant increase of neuroblasts in striatum 37 days after last injection<br>- No BrdU+/NeuN+ cells | Improvement in neurological score and adhesive tape removal test | Schavitz *et al.*, 2007 |

Table 2. Growth factors induce SVZ neurogenesis in ischemic stroke.

## 4. NSCs transplant therapy: A new expectation

Neural transplantation is a promising strategy for treatment of several CNS pathologies that offers long-lasting improvement and the prospect of permanent cure. The most obvious possibility is to use neural transplantation as a technique for cell replacement therapy

whereby the cells would occupy the place or the function of dead or degenerated cells. Potential advantages to this approach may include greater control over cell fate, the ability to deliver any desired number of cells, and reduced risks associated with mitogen infusion. A number of different cell types have been considered for cell transplantation with goals ranging from replacement of host circuitry to delivery of neuroprotective or immunomodulatory compounds.

The majority of studies to date have shown relatively limited cell replacement from endogenous NSCs. Further, the technology for mobilizing endogenous NSCs is relatively new. In contrast, work has been in progress for decades to replace lost neural cells by transplantation of either fetal brain tissue or more recently, NSCs.

Recent studies have highlighted the enormous potential of cell transplantation therapy for stroke. In this branch of the chapter, we will describe the experimental trials that utilized NSCs in the MCAO model, which placed the stepping stone for the first human trials of NSCs transplant therapy.

Fetal brain tissue transplants have been shown to produce some recovery in animal models of stroke (Mattsson *et al.,* 1999; Nishino *et al.,* 2000; Riolobos *et al.,* 2001), but ethical considerations and a short supply of human fetal tissue limited this approach. As a result, a variety of cell types have been tested in stroke models, they include human bone marrow cells, human umbilical cord blood cells (Chen *et al.,* 2001a; Chen *et al.,* 2001b; Savitz *et al.,* 2002; Zhao *et al.,* 2002), rat trophic factor-secreting kidney cells (Mattsson *et al.,* 1999; Nishino *et al.,* 2000; Riolobos *et al.,* 2001; Savitz *et al.,* 2002), and immortalized cell lines such as the human neuron-like NT2N (hNT) cells (Borlongan *et al.,* 1998; Saporta *et al.,* 1999) and MHP36, an embryonic murine immortalized neuroepithelial cell line (Modo *et al.,* 2002; Veizovic *et al.,* 2001). In spite of the vast types of transplanted cells employed, they yet need to demonstrate a significant behavioral recovery in animal models of stroke and a long-lasting survival of the grafted tissue (Table 3).

From the previously mentioned cell types employed for transplant, human cells that have been used in these studies fall into 3 categories: A) Neural stem/progenitor cells (NPCs) cultured from fetal tissue; B) immortalized neural cell lines, hematopoietic/endothelial progenitors and stromal cells isolated from bone marrow, umbilical cord blood, peripheral blood or C) adipose tissue. Even though transplanted human cells have shown promise, other sorts of cells have arisen to address the need for a quintessential cell source for transplant therapy.

Amongst them, NSCs have been proposed as a potential source of new cells to replace those lost due to central nervous system injury such as stroke, as well as a source of trophic molecules to minimize damage and promote recovery in clinical trials.

In the background of this imperative clinical need, hundreds of studies have recently published the therapeutic potential of either endogenous or transplanted NSCs in laboratory models of stroke. To their advantage, NSCs have the capacity to respond actively to their environment, migrate to areas of injury, and secrete neuroprotective compounds. Such properties may afford them therapeutic potential both in the acute phase and at later time points when the employment of conventional medical therapies would no longer be effective.

NSCs can be isolated from many regions of the CNS of embryonic as well as adult mammals. As mentioned in other section of the chapter, they can be propagated in culture in the presence of EGF and/or FGF-2 as proliferative clusters of cells termed neurospheres. Recent studies have demonstrated that rather than being homogeneous aggregates of stem cells, neurospheres actually represent a heterogeneous collection of cells including true stem cells, committed progenitors, and differentiated progeny. This is in contrast to embryonic stem cells (ESCs), which in the presence of appropriate signaling molecules can be maintained as a relatively homogeneous population of stem cells. NSCs also differ from ESCs in terms of the variety of neurons they can generate. Profiles of NSCs gene expression tend to point to NSCs expanded as neurospheres in EGF and FGF-2 as adopting a forebrain profile.

Consistent with this, attempts to differentiate NSCs into cells from other regions of the CNS, such as dopaminergic neurons, cerebellar Purkinje cells, or motoneurons have in most cases been unsuccessful. Nevertheless, NSCs can be successfully differentiated into representative cell types in parts of the brain most commonly affected by stroke, such as cortical projection neurons (Englund *et al.,* 2002), interneurons (Scheffler *et al.,* 2005) and hippocampal pyramidal neurons (Corti *et al.,* 2005). Retrograde labeling, synaptic integration, and action potential generation from NSCs-derived neurons has been demonstrated *in vivo* (Englund *et al.,* 2002).

Given the fact that NSCs have the capability to differentiate into neurons (Kelly *et al.,* 2004; Song *et al.,* 2002a; Song *et al.,* 2002b), astrocytes (Eriksson *et al.,* 2003; Herrera *et al.,* 1999; Winkler *et al.,* 1998), oligodendrocytes (Pluchino *et al.,* 2003; Yandava *et al.,* 1999), and perhaps endothelium (Wurmser *et al.,* 2004), advocates that conception that NSCs should be capable of replacing most of the cell types affected by an ischemic injury.

### 4.1 From theory to practice: NSCs transplant in rodent models of stroke

Actual results in preclinical studies, however, have been quite varied. NSCs, including human, can clearly survive after transplantation, have a tendency to migrate toward areas of infarct (Kelly *et al.,* 2004), and can generate functional neurons (Englund *et al.,* 2002) that may form connections with host cells (Park *et al.,* 2002). Although several studies have found NSCs to predominantly differentiate into glia after transplantation into normally non-neurogenic regions (Eriksson *et al.,* 2003; Herrera *et al.,* 1999; Winkler *et al.,* 1998), robust neural differentiation has been observed after transplantation of cells cultured on laminin (Wu *et al.,* 2002; Yan *et al.,* 2007).

Most studies have not observed substantial changes in infarct size after NSCs transplantation (Kelly *et al.,* 2004; Pollock *et al.,* 2006); however, neuroprotective (Lee *et al.,* 2007; Ourednik *et al.,* 2002) and immunomodulatory (Fujiwara *et al.,* 2004; Pluchino *et al.,* 2005) effects of NSCs in addition to their potential for at least some cell replacement (Sinden *et al.,* 1997), have collectively yielded beneficial effects in multiple animal models of neurodegeneration and brain injury, including stroke (Chu *et al.,* 2004; Pollock *et al.,* 2006; Sinden *et al.,* 1997).

NSCs transplantation enhances endogenous cell proliferation in the SVZ and promotes angiogenesis in the peri-infarct zone of adult rats, even if it is performed in the acute phase of ischemic injury. In addition, this transplanted NSCs managed to survive, migrate,

differentiate, and also induce improvement in neurological functions (Zhang *et al.,* 2009a, 2009b; Zhang *et al.,* 2010). Grafted NSCs enhanced the number of BrdU-positive cells in ischemic ipsilateral SVZ at 7 days after transplantation, an effect that persisted to at least 14 days post-transplantation. These results revealed that NSCs transplantation increases cell proliferation in the SVZ and promotes angiogenesis in the peri-infarct zone after focal cerebral ischemia in adult rats. The reason that grafted NSCs increase endogenous NSPCs proliferation may be due to the production of certain growth factors or repression of inflammation and apoptosis.

In addition to promote proliferation, it has been shown that human fetal striatum derived NSCs, transplanted as neurospheres, survive in stroke-damaged rat striatum, migrate toward the site of the injury, and differentiate into mature neurons in the absence of tumor formation (Darsalia *et al.,* 2007).

Evidence of cell migration to the site of ischemic injury from administration by various routes has been seen, including intravenous, intraarterial, and intraparenchymal brain injection. Migration potential may differ according to cell type and route, but this has not been systematically explored for most cell lines. Animal studies with a number of NSCs lines have shown evidence of cell survival and in some studies improvement in behavioral outcomes after focal ischemic injury (Bacigaluppi *et al.,* 2008; Borlongan *et al.,* 1998; Chu *et al.,* 2004; Ishibashi *et al.,* 2004; Jiang *et al.,* 2006; Modo *et al.,* 2002; Pollock *et al.,* 2006; Wei *et al.,* 2005).

In a follow-up work, Darsalia *et al.* showed that transplantation shortly after stroke (48 hours) resulted in better cell survival than did transplantation 6 weeks after stroke, but the delayed transplantation did not influence the magnitude of migration, neuronal differentiation, and cell proliferation in the grafts. Additionally, transplanting greater numbers of grafted NSCs did not result in a greater number of surviving cells or increased neuronal differentiation. They observed a substantial number of activated microglia 48 hours after the insult in the injured striatum, but reached maximum levels 1 to 6 weeks after stroke (Darsalia *et al.,* 2011). Their findings show that the best survival of grafted human NSCs in stroke-damaged brain requires optimum numbers of cells to be transplanted in the early post stroke phase, before the inflammatory response is established.

In an attempt to improve transplant survival and behavioral outcome, Jin *et al.* found that intralesional transplantation of nestin/Sox2-immunopositive neuronal precursor cells (NPCs) derived from BG01 human embryonic stem cells 3 weeks after distal MCAO in rats reduced infarct volume and improved behavioral outcome 4–9 weeks post-transplant (Jin *et al.,* 2010a). In another study, they found that the beneficial effects of transplantation occurred in both young adult (3-month-old) and aged (24-month-old) rats (Jin *et al.,* 2010b).

NPCs express many factors known to influence neurite plasticity and thus have the potential to enhance structural plasticity after stroke. With that notion in mind, Andres *et al.* decided to analyze the effects of transplanted NPCs on structural plasticity and axonal transport in the ischemic rat brain. They found that NPCs transplant one week after the ischemic event enhanced dendritic plasticity in both the ipsi- and contralesional cortex. Moreover, stem cell-grafted rats demonstrated increased corticocortical, corticostriatal, corticothalamic and corticospinal axonal rewiring from the contralesional side; with the transcallosal and corticospinal axonal sprouting correlating with functional recovery (Andres *et al.,* 2011).

With the advent and enhancement of molecular biology technology, tumor-derived neuronal tissues, including immortal teratocarcinoma-derived cells such as NT2N and hNT cells have been tested in stroke models (Bacigaluppi *et al.,* 2008; Borlongan *et al.,* 1998). Human fetal cortex cells have been immortalized by genetic modification (e.g. insertion of transcription factor genes including v-myc (Cacci *et al.,* 2007) and c-myc (Pollock *et al.,* 2006).

Irrespective of cell type or route of administration, cell survival has been limited. Quantification of cell survival is poorly reported. Reports, from the few studies that have attempted to quantify cell survival, range from 1% (Hicks *et al.,* 2009) to 30% (Darsalia *et al.,* 2007). Site of transplantation may be relevant to cell survival, with pathological evidence of 33.4 ± 6.1% viable cells when human fetal neural stem cells (hNSCs) were transplanted in non-ischemic tissue medial to the ischemic lesion (Kelly *et al.,* 2004). In contrast 30–50% of hNT cells have been suggested to survive in and around the ischemic tissue (Bliss *et al.,* 2006). Inflammation following ischemic stroke may aid cell migration (Belmadani *et al.,* 2006), but a negative correlation between cell survival and inflammatory response has also been observed (Kelly *et al.,* 2004), and may be a factor to consider when timing cell administration.

Although transplanted NSCs can recover some of the function lost after stroke, recovery has shown to be incomplete and restoration of lost tissue is minimal in most of the cases. The challenge set was to provide transplanted cells with matrix support in order to optimize their ability to engraft the damaged tissue. Bible *et al.* demonstrated that plasma polymerised allylamine (ppAAm)-treated poly(D,L-lactic acid-co-glycolic acid) (PLGA) scaffold particles can act as a structural support for neural stem cells injected directly through a needle into the lesion cavity using magnetic resonance imaging-derived coordinates. Upon implantation, the neuro-scaffolds integrated efficiently within the rat host tissue forming a primitive neural tissue. These work demonstrated that neuro-scaffolds could be a more advanced method to enhance brain repair. This study provides a substantial step in the technology development required for the translation of this approach.

Other cell sources employed for this purpose are the induced pluripotent stem (iPS) cell and mesenchymal stem cells (MSCs). iPS cells can be produced with high reproduction ability and pluripotency to differentiate into various types of cells, making them a feasible resource for transplantation, with the additional benefit of obtaining these cells from the same patient. On the other hand, MSC are multipotent stem cells that can differentiate into a variety of cell types.

In one study, undifferentiated iPS cells were transplanted into the ipsilateral striatum of a MCAO model of stroke; the transplanted iPS cells expanded and formed larger tumors in the post-ischemic brain compared with the control condition. iPS cells formed a tridermal teratoma (Kawai *et al.,* 2010). Despite this finding, iPS cells are still a hopeful alternative to provide neural cells for ischemic brain injury; however, tumor formation still needs to be prevented and controlled.

In another study that employed human bone marrow-derived MSCs (hBMSCs) found a significant recovery of behavior in the hBMSCs-treated rats beginning at 14 days after MCAO compared with the control animals. High levels of BDNF, neurotrophin-3 (NT-3), and VEGF were detected in the hBMSCs-treated brain, as well as an increased proliferation of neuronal progenitor cells in the SVZ (Bao *et al.,* 2011). This indicates that it is unlikely that

MSCs replaced the damaged tissue and more likely that they secreted trophic factors that promoted functional recovery after stroke.

Altogether, these findings, therefore, placed the stepping stone for direct clinical implications. However, evidence indicates that NSCs transplantation may protect the CNS from inflammatory damage via a "bystander" mechanism rather than by direct cell replacement (Martino & Pluchino, 2006).

### 4.2 Human trials with stem cells

Results of NSCs transplantation in ischemic stroke patients have not been reported, although in 2010 ReNeuron, a company in the United Kingdom received an approval to start a clinical trial using expanded NSCs. Therefore, we will witness the first clinical results of NSCs transplants. Nonetheless, the potential of precursor cells as an exogenous source for transplant therapy has been already assessed in some clinical trials (summarized in table 3).

Neural cells derived from an immortalized human teratocarcinoma cell line (NT2N cells) underwent two small clinical studies after showing improved behavioral outcomes in rats (Borlongan *et al.,* 1998). In the first human safety study (Stilley *et al.,* 2004), twelve patients who had suffered ischemic stroke on average 27 months earlier, received doses of between 2 and 6 million cells by direct injection into the basal ganglia. No cell related adverse events were reported. Some motor improvement was reported in around half the subjects at 6 months based on one clinical stroke scale and an increase in 18F-flurodeoxyglucose uptake on brain positron emission tomography (PET) scans was also reported, although of unclear significance. Subsequently Kondziolka and colleagues (Kondziolka *et al.,* 2005) reported on an open-label randomized phase II efficacy trial of 5–10 million NT2N cells including 14 actively treated subjects with stroke between 1 and 6 years earlier. Patients were randomized to receive cells with rehabilitation or rehabilitation alone in a 7:2 ratio and two ascending dose arms. Ischemic and hemorrhagic stroke formed half of the subjects each. No cell related adverse events were noted, and the major adverse events reported (seizure and subdural hematoma) were considered to be procedure-related. Neurological motor scores that were stable 6 months prior to surgery were reported to improve in 6/14 subjects by 6 months while in 4/14 subjects the scores deteriorated. There was no indication of any dose-related effects.

Savitz and colleagues used the intra-parenchymal route for delivery of fetal porcine derived cells in five patients, who suffered stroke 3–10 months prior (Savitz *et al.,* 2005). The study was halted after the fourth and fifth patients had worsening motor deficits and seizures. Whether cell or procedure related complications were the cause, it still remains unclear.

Mesenchymal stem cells have been administered intravenous in a controlled trial (Bang *et al.,* 2005) and autologous cells have been transplanted intraparenchymally in an open study (Suárez-Monteagudo *et al.,* 2009). Neither trial reported any cell or procedure related adverse events up to one year follow-up. In the latter study, clinical scores showed minimal and insignificant changes. Bang and colleagues reported improvement in one functional score of activities of daily living (Barthel index scores measured at 3, 6 and 12 months after cell therapy) but not in other clinical measures of outcome (modified-Rankin scale and National Institutes of Health Stroke Scale scores) and the number of subjects was small (5 actively treated and 25 controls).

| Cell Source | Stroke Area | Time period (after transplant) | Behavioral outcome | Nº of surviving cells | Reference |
|---|---|---|---|---|---|
| **NSCs transplant therapy in rodent models of stroke** | | | | | |
| Human Neuron-Like NT2N | MCAO | 24 weeks | 3-fold improvement of Passive Avoidance Test | NA | Borlongan *et al.*, 1998 |
| Rat Trophic Factor-Secreting Kidney Cells | MCAO | 20 weeks | 1 point augmentation in Prehensile Traction Test | NA | Mattsson *et al.*, 1999 |
| Human NT Neurons | MCAO | 12 weeks | 50% of behavioral improvement in functional tests | 24,217 ± 9,260 | Saporta *et al.*, 1999 |
| Human Umbilical Cord Blood Cells | MCAO | 5 weeks | Two points reduction of Modified Neurological Severity Score | 32 600 ± 1689 | Chen *et al.*, 2001a |
| Rat Bone Marrow Cells | MCAO | 2 weeks | 50% of behavioral improvement in functional tests | ~14% (Total of $4\times10^5$) | Chen *et al.*, 2001b |
| MHP36 Human Immortalized Cell Line | MCAO | 8 weeks | 2-Fold improvement in Water Maze Acquisition Test | ~7500 | Veizovic *et al.*, 2001 |
| MHP36 Human Immortalized Cell Line | MCAO | 4 weeks | 30% of behavioral improvement in functional tests | ~7500 | Modo *et al.*, 2002 |
| Human Bone Marrow Cells | MCAO | 2-6 weeks | 33.5 $\pm$ 8.7% of somatosensory asymmetries | NA | Zhao *et al.*, 2002 |
| Human NSCs neurospheres | Distal MCAO | 4 weeks | NA | 100,147 ± 28,944 | Kelly *et al.*, 2004 |
| CTX0E03 Human Neural Stem Cells | MCAO | 6-12 weeks | 50% of behavioral improvement in functional tests | NA | Pollock *et al.*, 2006 |
| iPS cells | MCAO | 7 weeks | None | Tumor of $50mm^3$ | Kawai *et al.*, 2010 |
| Human Bone Marrow-Derived Mesenchymal | MCAO | 4 weeks | Decrease of 4 points in the Modified Neurological Severity Scores | NA | Bao *et al.*, 2011 |
| **NSCs transplant therapy in stroke patients** | | | | | |
| **Cell Source** | **Stroke Area** | **Time period (after transplant)** | **Behavioral outcome** | **Nº of surviving cells** | **Reference** |
| Neural MSCs derived | MCA | 48 weeks | Significant improvement for 12 weeks in Barthel Index (30 points augmentation) | NA | Bang *et al.*, 2005 |
| Human Neuronal Cells | NA | 96 weeks | Improvement of 6.9 points in the European Stroke Scale at 24 weeks | NA | Kondziolka *et al.*, 2005 |

| Cell Source | Stroke Area | Time period (after transplant) | Behavioral outcome | Nº of surviving cells | Reference |
|---|---|---|---|---|---|
| Neural Fetal Porcine Cells | Basal Ganglia | 192 weeks | Improvement in speech, language and/or motor impairments in 40% of the patients | NA | Savitz *et al.*, 2005 |
| Bone Marrow Stem Cells | NA | 48 weeks | None | NA | Suárez-Monteagudo *et al.*, 2009 |

Table 3. Experimental and clinical NSCs transplant therapy in stroke.

### 4.3 The prospect of NSCs transplant therapy for stroke

NSCs transplantation therapy for stroke holds great promise. However, the mechanisms of recovery are not completely understood. It is very likely that more than one mechanism is involved in the processes of recovery, and it still remains to be answered.

To this end, some standardization of the basic research, especially for behavior, is needed so that direct comparisons can be made between studies. Furthermore, longer-term studies are required to determine whether the cell-enhanced recovery is sustained and also to determine the tumorigenic potential of the cells. Other challenges include ensuring appropriate characterization, manufacturing, and quality control of transplanted cells and rigorous testing of viral and adventitious agents. Clearly, more research is needed to understand the bidirectional interaction between the transplanted cells and the host to optimize the chances of success before proceeding to the clinic.

Although reconstructing normal brain circuitry following stroke via NSCs is not likely in the foreseeable future, and although great care must be taken to ensure safety before considering clinical trials, preliminary evidence supports the therapeutic potential of NSCs for treatment of ischemic brain injury in animal models.

Understanding the mechanism of action of human NPCs in the post-ischemic brain will be important for the successful translation of cell transplantation strategies to the clinic. For example, if modulation of host brain plasticity is a major human NPCs mechanism of action, this could dictate the best time to transplant cells after stroke; "network relearning" occurs within weeks of stroke and continues for several months, making it a good therapeutic target with a large time window of intervention. Furthermore, knowing what changes the human NPCs elicit in the brain offers useful surrogate indicators of transplanted cell activity.

## 5. Conclusions

Under normal and pathological conditions, the adult brain is able to preserve regions with regenerative potential. Current research of neurogenic niches is revealing their complex homeostatic process, but at the same time, is bestowing with expectation that unraveling the characteristics of the unique molecular environment of the SVZ and the understanding of the underlying mechanisms that regulate the creation of new neurons in the adult brain will allow us to manipulate NSCs to yield a significant number of neurons capable of integration into human functional brain circuitries that were damaged and improve the motor deficits secondary to stroke or neurodegenerative diseases.

Long-term studies reviewed here support the persistence of an attenuate plasticity process residing within the neurogenic SVZ and RMS throughout life, which can be modulated in major extent by the action of some growth factors. The employment of growth factors could circumvent the technical and ethical constraints by using stem cells for transplant therapy. In addition, autologous transplantation of NSCs expanded *in vitro* could also avoid these concerns, and for this reason, it can be considered as a promising alternative. The first transplant of NSCs in stroke patients is currently in progress.

Altogheter, the large body of evidence supports the manipulation of endogenous NSCs and employment of grafted stem cells as future treatments for acute and chronic stroke. In spite of current efforts, the effectiveness and safety of both approaches are still being developed. It is clear that further investigation is necessary before such methods can be applied for human treatment and in our opinion, successful cell therapy for stroke patients, is still in a distant future.

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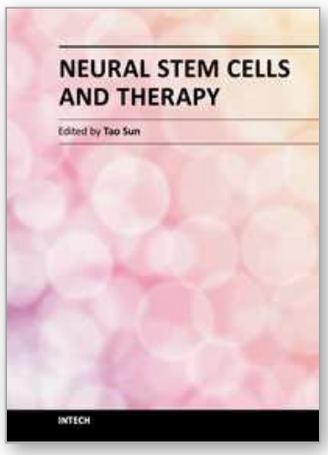


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This book is a collective work of international experts in the neural stem cell field. The book incorporates the characterization of embryonic and adult neural stem cells in both invertebrates and vertebrates. It highlights the history and the most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



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