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Roles of PDGF for Neural Stem Cells

BY

MIA ENARSSON



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Abstract

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Stem cells are endowed with unique qualities: they can both self-renew and give rise to new mature cell types. Central nervous system (CNS) stem cells can give rise to neurons and glia. What factors regulate stem cell fate decisions? Identifying signals that are involved in the regulation of CNS stem cell proliferation, survival, differentiation and migration is fundamental to the understanding of CNS development. In addition, this knowledge hopefully will contribute to more efficient therapies of CNS damages and diseases.

The focus of this thesis was to investigate mechanisms of CNS stem cell proliferation and differentiation. We have studied the role for platelet-derived growth factor (PDGF) in these cellular events both *in vitro* and *in vivo*. Previous reports have shown that PDGF are implicated in brain tumorigenesis and also supports neuronal differentiation of CNS stem cells. We have found that PDGF promotes survival and proliferation of immature neurons, thereby supporting neuronal differentiation. The intracellular Ras/ERK signaling pathway probably mediates the mitogenic activity of PDGF. In contrast, neuronal differentiation is not dependent on the Ras/ERK pathway. A genetic expression profile of stem cells during their differentiation was obtained. This microarray analysis suggests that PDGF-treated stem cells are at an intermediate stage between proliferation and differentiation. Furthermore, we generated transgenic mice that overexpress *Pdgf-b* in neural stem cells. Preliminary data indicate no signs of enhanced proliferation of immature neurons. Instead, increased apoptosis was detected in the developing striatum.

The results presented in this thesis show how CNS stem cells are regulated by PDGF. PDGFs are widely expressed in the developing CNS and also in some brain tumors, which are thought to arise from CNS stem cells. Thus, this knowledge may contribute to an increased understanding of brain tumorigenesis in addition to normal CNS development.

Keywords: CNS, stem cells, neuron, PDGF, proliferation, differentiation, ERK, microarray, transgenic mouse

Mia Enarsson, Department of Medical Biochemistry and Microbiology, Box 582, Uppsala University, SE-75123 Uppsala, Sweden

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To life and music!

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I Erlandsson A., Enarsson M., Forsberg-Nilsson K. (2001) Immature neurons from CNS stem cells proliferate in response to platelet-derived growth factor. *J Neurosci* 21: 3483-3491.
- II Enarsson M., Erlandsson A., Larsson H., Forsberg-Nilsson K. (2002) Extracellular signal-regulated protein kinase signaling is uncoupled from initial differentiation of central nervous system stem cells to neurons. *Mol Cancer Res* 1: 147-154.
- III Demoulin J-B.*, Enarsson M.*, Larsson J., Adams B., Heldin C-H., Forsberg-Nilsson K. Analysis of gene expression in neural stem cells during proliferation and differentiation. Manuscript.
- IV Enarsson M., Zhang X-Q., Forsberg-Nilsson K. Overexpression of PDGF-B in neural stem cells leads to increased apoptosis in the developing striatum. Manuscript.

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* These authors contributed equally to this work.

Contents

Background	9
Introduction	9
Stem cells.....	9
Stem cell characteristics.....	9
Plasticity of stem cells.....	10
Stem cell therapy	11
Central nervous system	13
Development.....	13
Embryonic neural stem cells.....	14
Adult neural stem cells.....	15
Radial glia - are they neural stem cells?	15
Mature cells in the CNS: neurons and glial cells	16
Regulation of CNS stem/progenitor cell fates.....	17
Proliferation	17
Differentiation	18
Fibroblast growth factor (FGF).....	20
Ligands and receptors.....	20
FGF and CNS development	20
Platelet-derived growth factor (PDGF)	22
Ligands and receptors.....	22
PDGF and CNS development	23
PDGF knockouts	24
FGF and PDGF receptor signaling	25
Receptor tyrosine kinases.....	25
Ras/MAP kinase signaling pathway	26
Cancer	26
Transforming activity of PDGF	26
PDGF transgenic mice	27
Stem cells and brain tumors	28
Is a stem cell really a stem cell?	28
Neural stem cell culture systems.....	28
Cell-specific molecular markers	30
Present investigation	33
Results and discussion	33

Immature neurons from CNS stem cells proliferate in response to PDGF (paper I)	33
ERK signaling is uncoupled from initial differentiation of CNS stem cells to neurons (paper II)	35
Analysis of gene expression in neural stem cells during proliferation and differentiation (paper III)	36
Overexpression of PDGF-B in neural stem cells leads to increased apoptosis in the developing striatum (paper IV)	37
Future perspectives	40
Populärvetenskaplig sammanfattning.....	42
Acknowledgements.....	44
References	47

Abbreviations

BDNF	Brain-derived neurotrophic factor
bHLH	Basic helix-loop-helix
BLBP	Brain lipid-binding protein
BMP	Bone morphogenetic protein
BrdU	Bromodeoxyuridine
CCg	Cystatin C glycosylated
cDNA	Complementary deoxyribonucleic acid
CNP	Cyclic nucleotide phosphodiesterase
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
E	Embryonic day
EGF	Epidermal growth factor
ERK	Extracellular signal-regulated kinase
ES	Embryonic stem (cell)
EST	Expressed sequence tag
FACS	Flourescence-activated cell sorter
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FRS	FGF receptor substrate
GABA	-aminobutyric acid
GalC	Galactocerebroside
GDP	Guanosine diphosphate
GFAP	Glial fibrillary acidic protein
GLAST	Glutamate transporter astrocyte-specific
GTP	Guanosine triphosphate
HSPG	Heparan sulfate proteoglycan
IGF	Insulin-like growth factor
LGE	Lateral ganglionic eminence
LIF	Leukemia inhibitory factor
MAG	Myelin-associated glycoprotein
MAP2	Microtubule associated protein 2
MAPK	Mitogen activated protein kinase
MASH	Mammalian achaete-scute homolog
MBP	Myelin basic protein

MEK	Mitogen-induced extracellular kinase
NF	Neurofilament
Ngn	Neurogenin
NSC	Neural stem cell
NSE	Neuron-specific enolase
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PI-3-K	Phosphatidylinositol-3-kinase
PINK	PTEN-induced protein kinase
PLP	Proteolipid protein
PNET	Primitive neuroectodermal tumor
PNS	Peripheral nervous system
PTB	Phosphotyrosine binding
PTEN	Phosphatase and tensin homolog
RNA	Ribonucleic acid
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcriptase polymerase chain reaction
SH	Src homology region
Shh	Sonic hedgehog
T3	Triiodothyronine
TGF	Transforming growth factor
TH	Tyrosine hydroxylase
TMSTDE	Transmembrane protein tumor differ- entially expressed
TRAIL	Tumor necrosis factor-related apoptosis-inducing factor
TUNEL	TdT-mediated X-dUTP nick end labeling

Background

Introduction

Stem cells possess a unique quality: they can form many different mature cell types of the body. Since there is a lack of efficient therapies for many diseases today, this capacity of stem cells is of course of great clinical value. A lot of attention has been given to stem cells in the central nervous system (CNS) during the last several years. These cells can give rise to new neurons as well as other cell types in the CNS, both during development and in adulthood. What regulates the cell fate choice of a stem cell? Identification of signals that are involved in the regulation of CNS stem cell proliferation, survival, differentiation and migration is fundamental to the understanding of CNS development. In addition, this knowledge will hopefully contribute to more efficient therapies for CNS pathologies, such as Parkinson's disease and brain tumors.

Stem cells

Stem cell characteristics

A stem cell is an immature cell that is capable to self-renew, i.e. divide symmetrically and make copies of itself. A second feature of a stem cell is that it has a broad differentiation potential, which means that it can give rise to several mature cell types in the body (Gage, 2000; Weissman, 2000). When a stem cell divides asymmetrically, it gives rise to a new stem cell plus a more differentiated cell (Figure 1). Stem cells can be subdivided into different groups, according to their differentiation potential: totipotent, pluripotent, and multipotent (Baizabal et al., 2003). The fertilized egg is a totipotent stem cell with a differentiation potential to give rise to all cell types in the embryo as well as the placenta. A pluripotent stem cell has the ability to form cells of different lineages, e.g. stem cells in the early embryo that can develop into all different cell types within the body. The third group of stem cells, multipotent stem cells, refers to cells that can give rise to cell

types belonging to a common specific cell lineage, e.g. hematopoietic, muscle, and neural stem cells. Multipotent stem cells are called somatic stem cells, from the Greek word for 'body', soma. Some somatic stem cells not only exist during embryo development, but also reside into adulthood in

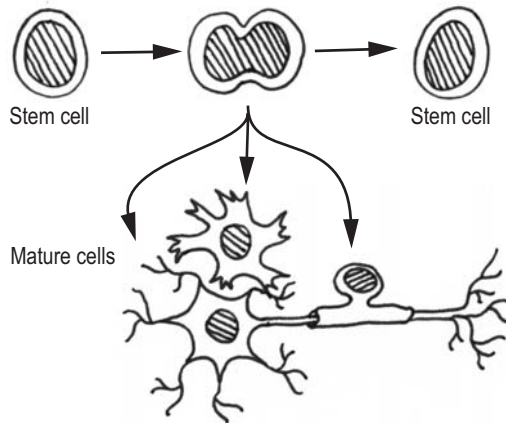


Figure 1. A stem cell can give rise to a new stem cell as well as different mature cell types.

many organs. The hematopoietic system and the skin are examples of organs that need many stem cells for the continuous supply of new mature cell types. Stem cells reside in other organs as well, such as in the nervous system and the kidney, but these organs do not need to form new cells at the same rate. When stem cells differentiate, they go through different maturation levels before they reach their final mature and functional cell type. These mid-stage cells are called progenitor cells.

Plasticity of stem cells

Stem cells from the early embryo can be grown in the laboratory, *in vitro*, and are called embryonic stem cells (ES cells) (Smith, 2001). These cells are pluripotent and can differentiate into all somatic cells found in the adult organism, i.e. they have a great plasticity. Recent data suggest that ES cells also can make germ cells *in vitro* (Geijsen et al., 2004; Hubner et al., 2003; Toyooka et al., 2003). Today, much effort is invested in developing efficient protocols for generation of organ-specific cell types from ES cells. The pivotal aim is to make it possible to transplant these specific cells to patients and thereby recover damaged tissues caused by a variety of diseases.

A current controversy, which has been the subject of much recent debate, is the plasticity of multipotent somatic stem cells. Some reports assert that somatic stem cells are not restricted to give rise to the mature cells of the organ they reside in but also to other cell types in body, a process called transdifferentiation (Bjorklund and Svendsen, 2001; Liu and Rao, 2003; Tsai et al., 2002). These studies suggest that somatic stem cells from different organs are similar to each other and that it is mostly the environment that regulates the fate of a stem cell, and not an intrinsic differentiation program. Accordingly, this means that adult neural stem cells not necessarily have to become brain cells, but can develop into for example a blood cell if they are

placed in a “blood environment” (Bjornson et al., 1999). Is this a relevant phenomenon? During early embryo development, neuronal cells are generated from a structure called the neural plate, which can also give rise to cartilage, bone and smooth muscle cells (Gilbert, 2000). Thus, different somatic cells can originate from a common germ layer *in vivo*, indicating that they might be related and possibly can adopt each other's characteristics also *in vitro*. A number of different somatic stem/progenitor cells have been reported to transdifferentiate: bone marrow cells that have given rise to neural cells (Brazelton et al., 2000; Kopen et al., 1999; Mezey et al., 2000), muscle cells (Ferrari et al., 1998; Gussoni et al., 1999), and myocardium (Orlic et al., 2001); muscle precursor cells generating adipocytes (Hu et al., 1995); liver cells developing into pancreatic cells (Overturf et al., 1997); skin stem cells forming neurons (Toma et al., 2001); and, in addition to hematopoietic cells, neural stem cells transdifferentiating into muscle cells (Clarke et al., 2000; Galli et al., 2000a).

Other reports reject the transdifferentiation hypothesis and suggest the fate change is due to cell fusion. In two studies somatic stem cells have been co-cultured with ES-cells, resulting in fused tetraploid cells expressing specific cell markers of both cell types (Terada et al., 2002; Ying et al., 2002). These *in vitro* experiments were recently verified *in vivo*, when irradiated mice were transplanted with bone marrow-derived cells. Cell fusion between transplanted cells and recipient cells could be detected in the brain (cerebellum), heart, and liver (Alvarez-Dolado et al., 2003). Furthermore, Grompe and colleagues showed that their previous report (Lagasse et al., 2000), suggesting transdifferentiation of hematopoietic stem cells into hepatocytes, was actually a result of cell fusion (Wang et al., 2003). Cell fusion does usually not occur in most organs, but is a normal event in liver and muscle. Therefore, special care must be taken when studying hepatocytes and muscle cells regarding transdifferentiation and cell fusion. Further efforts in this area are needed to clarify the relevance of transdifferentiation and cell fusion. The clonal differentiation of the somatic stem cells must be addressed to generate convincing data to support or refute the concept of stem cell plasticity.

Stem cell therapy

Stem cells have a great therapeutic potential. These cells may provide an approach to rebuild damaged tissues, thereby restoring normal function for the patient. At present, transplantation of hematopoietic stem cells is used in the clinic to treat e.g. leukemia patients. In the future, hopefully several other diseases, such as neurodegenerative diseases and diabetes, will be cured by cell transplantation.

The diverse differentiation repertoire of ES cells makes them ideal candidates for the generation of tissues for transplantation therapies. The hope is that ES cells can be used to produce new tissue-specific cells, followed by

transplantation of these to patients with certain damages and diseases. Such therapy has already worked in mice. For example, ES cell-derived neurons and oligodendrocytes have been shown to restore functions when injected into damaged rodent central nervous systems (Barberi et al., 2003; Brustle et al., 1999; Liu et al., 2000). However, data from transplantation experiments performed on mice cannot be directly transferred to humans. Transplantation of ES cell-derived cells may cause rejection of the cell transplant. To overcome the problem of host rejection, human ES cells might have to be genetically modified. This can be achieved by exchanging the egg cell nucleus with a somatic cell nucleus from the patient, so called therapeutic cloning (Figure 2) (Colman and Kind, 2000). ES cells are then isolated from the modified blastocyst (early-stage embryo) and induced to differentiate to the desired cell type, followed by transplantation. Thus, the immune system of the patient will recognize the transplanted cells as the patient's own.

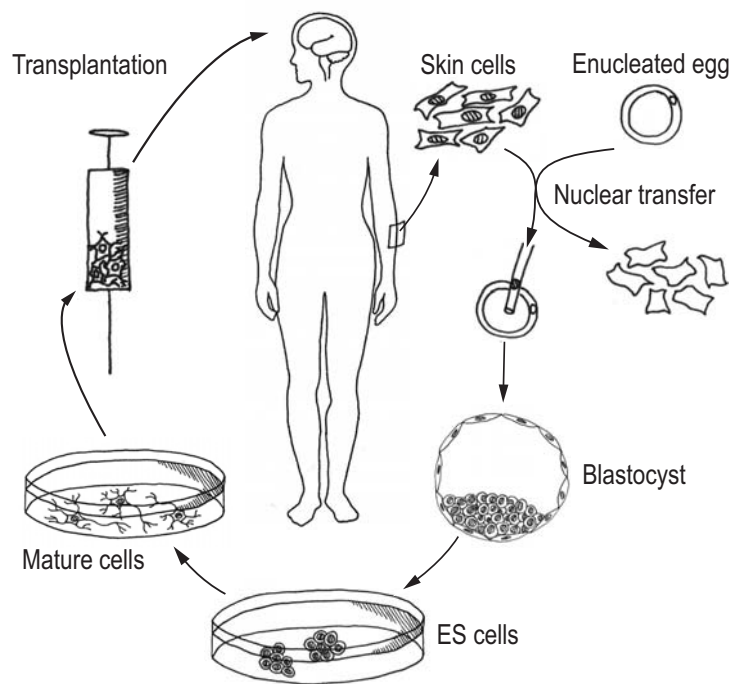


Figure 2. *The principle of therapeutic cloning. The nucleus of a somatic stem cell, e.g. skin cell is taken from the patient and transferred to an enucleated fertilized egg. ES cells, isolated from the blastocyst, are then directed to develop towards the specific cell type needed to regenerate the damaged tissue of the patient. Subsequently, the immune system will recognize the cell transplant as the patient's own cells.*

Another problem that has to be solved before ES cells can be used in the clinic is that ES cells can develop into teratomas, a certain type of tumors (Hogan et al., 1994). If the transplanted cells contain a fraction of undifferentiated ES cells, these cells might cause tumors. In conclusion, caution should be taken when transplanting cells whose *in vivo* behavior is not fully understood. To reach this therapeutical goal, much additional knowledge has to be gained regarding the regulation of stem cell survival, proliferation, migration and differentiation.

In the following chapters, stem cells from the central nervous system (CNS) will be discussed. Neural stem cells might have a therapeutical role for different damages and diseases affecting the brain and the spinal cord, such as Parkinson's and Alzheimer's disease, stroke, and spinal cord injury.

Central nervous system

Development

Mammalian development begins with a totipotent stem cell: the fertilized egg. A few days after fertilization, a blastocyst is formed. It consists of the trophoectoderm, which later gives rise to the placenta, and the inner cell mass. Pluripotent embryonic stem (ES) cells are the *in vitro* counterpart of the inner cell mass. The inner cell mass forms the epiblast, which generates the whole embryo. During gastrulation, the epiblast develops into the three embryonic germ layers: endoderm, mesoderm, and ectoderm. The endoderm ultimately gives rise to e.g. the lining of many of the internal organs, whereas the mesoderm forms structures such as the skeletal bones and the muscles. From the ectoderm arise the skin and the nervous system. The nervous system is divided into the central nervous system (CNS) and the peripheral nervous system (PNS). CNS consists of the brain and the spinal cord, and the PNS contains all nerves and ganglia outside the CNS (Hogan et al., 1994; Kandel et al., 2000; Kaufman, 1992; Kaufman and Bard, 1999).

The development of the CNS begins when a large sheet of ectodermal cells forms the neural plate (around 3 weeks of gestation in humans and embryonal day 7.5 in mice). During a process called neurulation, the neural plate begins to fold into a tubular structure, the neural tube (Figure 3). The entire central nervous system develops from the walls of the neural tube. Some neural ectoderm is pinched off during neurulation, forming the neural crest, just lateral to the neural tube. The neurons with cell bodies in the peripheral nervous system derive from the neural crest. The anterior end of neural tube differentiates to form three primary vesicles, forebrain, midbrain and hindbrain, and the posterior end forms the spinal cord. In the ventricular zone, closest to lumen of the neural tube, stem cells divide according to a

strictly controlled temporal and spatial schedule. The postmitotic progeny start to migrate from the ventricular surface to the periphery, guided by radial glial cells, and the diverse set of neurons and glial cells in the brain and spinal cord is produced (Hogan et al., 1994; Kandel et al., 2000; Kaufman, 1992; Kaufman and Bard, 1999).

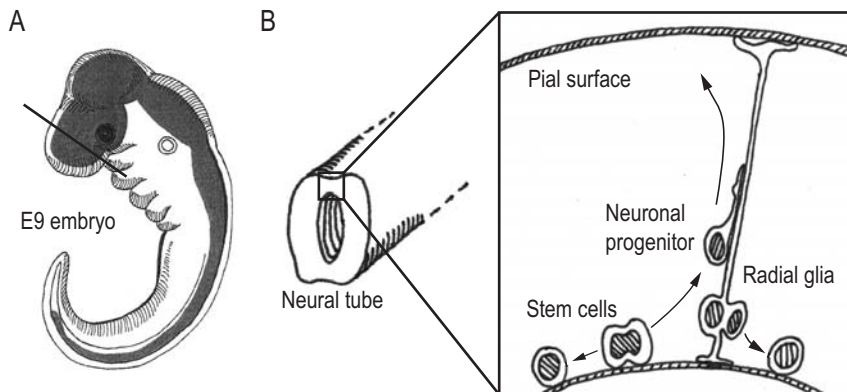


Figure 3. A. An embryonic day 9 mouse. The line indicates the location of the neural tube cross-section illustrated in B. B. In the ventricular zone, closest to the lumen of the neural tube, stem cells divide and start to differentiate. Newborn progenitor cells migrate along radial glia fibers towards the pial surface and their final destinations. Radial glia were recently shown to have stem cell characteristics, being capable of generating neurons during development and astrocytes postnatally.

Embryonic neural stem cells

Neural stem cells (NSCs) appear during neural plate formation and possibly constitute the major cell type of the early ectoderm (Davis and Temple, 1994; Temple, 2001). In the neural tube, neural stem cells respond to signals that determine their anterior-posterior and dorsal-ventral axis organization (Altmann and Brivanlou, 2001). Already at E8.5 in mouse, the neural tube is divided into distinct structures, and each region expresses its own specific transcription factor code, the "positional identity" (Altmann and Brivanlou, 2001; Wolpert, 1994). Thus, neural stem cells in the early neural tube are not identical, but have their own regional specificity.

In the ventricular zone of the neural tube, neural stem cells divide extensively, followed by differentiation into a diverse set of neural cells (Figure 3B). The earliest differentiation occurs around embryonic day 11 (E11) in mouse neural tube, starting with the generation of neurons (neurogenesis) (Qian et al., 2000; Temple, 2001). Newborn neurons, or neural progenitor cells, start to migrate away from the ventricular zone. Radial glial cells guide them to the surface of the developing brain. This phenomenon, called radial

migration, establishes the neuronal layers of e.g. the cerebral cortex. It occurs in an inside-out manner, with the earliest-generated neurons positioned in the deepest layers and later-generated neurons occupying the superficial layers (Rakic, 1972; Rakic, 1974). In later stages of CNS development, around E17 in mouse, neural stem cells start to form the other mature cells in the CNS: astrocytes and oligodendrocytes (gliogenesis) (Levers et al., 2001; Qian et al., 2000; Temple, 2001). There are however indications that glial precursor cells arise as early as E10-12.5 in rodents - the time when neurogenesis is initiated (Chandross et al., 1999; Zhou et al., 2000).

Adult neural stem cells

An old dogma establishes that generation of neurons only exists during embryo and postnatal development. However, a decade ago evidence came (Lois and Alvarez-Buylla, 1993; Reynolds and Weiss, 1992; Reynolds and Weiss, 1996) that supported previous data, suggesting the emergence of new neurons in adult mice (Altman and Das, 1965). Furthermore, Eriksson and colleagues demonstrated that neurogenesis takes place also in the human adult brain (Eriksson et al., 1998). Adult neural stem cells reside at restricted niches, primarily in the dentate gyrus of the hippocampus and in the ventricular/subventricular zone adjacent to the lateral ventricles (Gage, 2002; Momma et al., 2000). The identification of the true stem cell in the adult brain has been debated. Ciliated ependymal cells lining the lateral wall (Johansson et al., 1999), astrocytes in the subventricular zone (Doetsch et al., 1999; Laywell et al., 2000; Seri et al., 2001), and radial glia (Alvarez-Buylla et al., 1990) are strong candidates in these discussions. The functional relevance of the *de novo* generation of neurons in the adult has not yet been defined, but a role in learning and olfactory function has been suggested from studies in canaries and mice (Goldman and Nottebohm, 1983; Gould et al., 1999; Rochefort et al., 2002).

Could endogenous adult NSCs participate in neuron regeneration upon accidental or pathological damage of the CNS? Some reports have shown that precursor cells in the neurogenic centers start to proliferate and migrate in response to experimental lesions in rodent's brain, including stroke, ischemia, and epileptic seizures (Arvidsson et al., 2002; Nakatomi et al., 2002; Parent et al., 2002). Nonetheless, it is clear that damage to the CNS is not matched by the ability of endogenous precursor cells to replace lost cells.

Radial glia - are they neural stem cells?

Early in the neural tube development, before the generation of neurons has started, radial glial cells appear. They show a bipolar morphology, extending across the entire radial axis of the neural tube, from the ventricular zone out to the pial surface (Figure 3B). Traditionally, radial glial cells were consid-

ered as glial precursor cells due to their astroglial traits and the fact that they later during CNS development produce astrocytes. Their role was thought to be to solely act as guides for newborn neurons, migrating towards their final destinations (Rakic, 1972). Recent evidence obtained *in vitro* (Malatesta et al., 2000) and *in vivo* (Noctor et al., 2001; Noctor et al., 2002), however, reveal that a large subset of radial glia has characteristics of neural progenitor cells. In some neural tube regions (e.g. cerebral cortex) radial glia progenitors seem to participate in nearly all neurogenesis during development (Malatesta et al., 2003). Interestingly, late in forebrain development radial glial cells produce astrocytes, a cell type that has been suggested to be a prospective neural stem cell in the adult brain.

Mature cells in the CNS: neurons and glial cells

Neurons and glia constitute the mature cells in the brain and the spinal cord (Figure 4) (Kandel et al., 2000). The primary purpose of the brain is to acquire, coordinate, and disseminate information about the body and its environment. To perform this task, neurons have evolved sophisticated means of generating electrical and chemical signals. The mammalian central nervous system contains a diverse set of neurons, varying in shape, size, and functions. The stereotypical image of a neuron is that of a stellate cell body with broad dendrites emerging from one pole and a fine axon emerging from the opposite pole. The branched dendritic tree receives inputs from multiple surrounding neurons.

The axon does not usually branch until it reaches its target. In contrast to the dendrites, the axon is frequently myelinated. The myelin is produced by oligodendrocytes in the CNS and functions to facilitate the neurotransmission, increasing the signaling rate of neurons.

The glial cell lineage consists of oligodendrocytes and astrocytes. The term glia is derived from the Greek word for glue, giving the impression that these cells only fill the spaces between the neurons. This is however not the whole truth, as they contribute to brain function by multiple actions, such as insulating, supporting, instructing, and nourishing neighboring neurons. Astrocytes are also important regulators of the blood-brain-barrier (Kandel et al., 2000). Recently, astrocytes were shown to be able to induce a neuronal

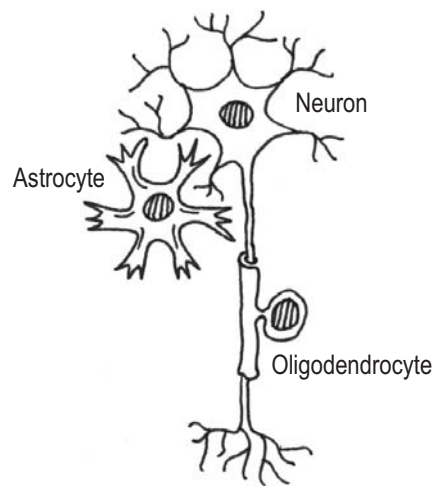


Figure 4. Neurons, astrocytes and oligodendrocytes are mature cells in the central nervous system

phenotype on adult stem cells from mice, both as a result of cell-cell contact and by secreted factors (Song et al., 2002). This role in fate specification is unexpected because, during development, neurons are generated previous to most of the astrocytes.

Regulation of CNS stem/progenitor cell fates

One key issue in developmental neurobiology is to understand how the complex brain orchestrates proliferation, differentiation, and migration of various cell types in a precise temporal and spatial order. Which factors regulate the transition from a multipotential, self-renewing stem cell to a more specified progenitor? This question has only partly been answered, and a lot of questions remain. *In vivo*, the generation of neurons precedes that of astrocytes and oligodendrocytes. The fate choice is highly regulated, to some extent by cell intrinsic signals, such as expression of specific transcription factors (Schuurmans and Guillemot, 2002). Other factors that are critical for cell regulation are cell-cell contacts (Dutton and Bartlett, 2000; Tsai and McKay, 2000), cell-extracellular matrix interactions (Brocco and Panzetta, 1999; Frost et al., 1999; Jacques et al., 1998; Testaz et al., 1999), and delivery and concentration of multiple soluble factors in the external microenvironment (Arsenijevic, 2003; Burrows et al., 1997; Sommer and Rao, 2002). In the following sections, focus will be on factors that influence proliferation and differentiation of rodent neural stem/progenitor cells *in vitro* (Figure 5).

Proliferation

Neural stem/progenitor populations from different neural tube regions respond differentially to cytokines and growth factors, and they also undergo changes in their responsiveness to mitogenic factors as embryonic development proceeds. Self-renewal occurs in the presence of mitogens, such as fibroblast growth factor (FGF) and epidermal growth factor (EGF), both *in vitro* and *in vivo* (Gage, 2000; Kilpatrick and Bartlett, 1995; Maric et al., 2003; McKay, 1997; Reynolds and Weiss, 1996; Santa-Olalla and Covarrubias, 1995). FGF-2 and EGF are the most commonly used mitogens for neural stem/progenitor cell cultures.

Several other important mitogenic factors have been reported as well. Transforming growth factor- β (TGF- β) signal through the EGF receptor and can mediate neurosphere formation of E13.5 murine NSCs (Santa-Olalla and Covarrubias, 1995). Sonic hedgehog (Shh) signaling is involved in numerous processes during development, including dorsal-ventral patterning in the neural tube, as well as acting as a mitogen for both embryonic and adult neural stem cells (Lai et al., 2003; Machold et al., 2003; Rowitch et al., 1999; Ruiz i Altaba et al., 2002). During early embryogenesis, leukemia

inhibitory factor (LIF) (Carpenter et al., 1999) and Notch (Lewis, 1998) are involved in maintaining neural stem cells in a proliferative state. None of these factors are however pure mitogens for neural stem cells, as they also contribute to lineage-specification during later embryogenesis.

During neural tube development, two distinct neural stem cell populations appear sequentially: NSCs from the early neural tube require FGF-2 for proliferation, whereas NSCs from later developmental stages proliferate with FGF-2 and/or EGF (Tropepe et al., 1999). Recently, "early" NSC and "late" NSC have been found along the entire neural tube, but, as development advances, the early NSCs become more restricted to the posterior part of the neural tube (Santa-Olalla et al., 2003). The suggested mechanism behind the change in growth factor response is that FGF-2 up-regulates receptors for EGF and, consequently, embryonic FGF-2-responsive cells later acquire EGF responsiveness (Lillien and Raphael, 2000; Santa-Olalla and Covarrubias, 1999).

Both FGF-2 and EGF need additional factors for their mitogenic actions. FGF-2 requires the glycosylated form of Cystatin C (CCg) for mediating its mitogenic activity *in vitro* and to stimulate neurogenesis *in vivo* (Taupin et al., 2000). In addition, FGF-2 and also EGF are dependent on insulin-like growth factor 1 (IGF-1) (Arsenijevic et al., 2001). If the endogenous IGF-1 activity is blocked, the mitogenic signaling by FGF-2 is completely abolished (Drago et al., 1991). In that context, it is important to note that neural stem cells cultured in serum-free medium require high concentrations of insulin for survival. Insulin and IGF-1 display some cross-affinity for each other's receptors. Thus, insulin can act in a limited role as a growth factor and IGF-1 can exhibit metabolic effects.

Differentiation

The fate choice of a neural stem cell is regulated by a complex machinery *in vivo*. Proliferating rodent neural stem cells *in vitro* can be induced to differentiate simply by withdrawal of the mitogen, and consequently give rise to different proportions of neurons, astrocytes, and oligodendrocytes (Gritti et al., 1996; Johe et al., 1996). The *in vitro* differentiation can be directed towards specific cell types by adding different soluble factors, such as neurotransmitters, neurotrophins, cytokines and growth factors to the culture medium (Johe et al., 1996; Panchision and McKay, 2002; Sommer and Rao, 2002). Neural stem/progenitor cells from different ages (early - late development, or adult) and species possess different response to these factors.

Bone morphogenetic proteins (BMP) 2 and 4 have been shown to promote differentiation of neural stem cells into neurons in mid-gestation CNS precursors (Li et al., 1998) and astrocytes in late embryonic or adult CNS precursors (Gross et al., 1996). These different actions by BMPs seem to be dependent on the levels of the proneural basic helix-loop-helix (bHLH) tran-

scription factor, neurogenin1 (Ngn1). When high levels of Ngn1 are expressed in the cell neurogenesis is promoted, whereas gliogenesis occurs at low levels of Ngn1 (Sun et al., 2001).

Platelet-derived growth factor (PDGF) can support neuronal differentiation of rodent neural stem cells, when FGF-2 is absent (Johe et al., 1996; Williams et al., 1997). PDGF does not instructively regulate the fate choice, but rather acts as a survival and proliferation factor for immature neurons (Erlandsson et al., 2001).

Differentiation of rodent E14.5 neural stem cells into astrocytes can be efficiently promoted by the cytokines ciliary neurotrophic factor (CNTF) and LIF (Bonni et al., 1997; Johe et al., 1996), except during earlier stages of CNS development (Molne et al., 2000). The action of CNTF is mediated by a direct, instructive mechanism. In resemblance to LIF, Notch also switches from promoting stem cell proliferation during early embryogenesis to induce astrocytic differentiation at later stages (Morrison et al., 2000). Oligodendrocytic differentiation can be augmented by the thyroid hormone triiodothyronine (T3) (Johe et al., 1996) and by IGF-1 (Barres et al., 1992; McMorris and Dubois-Dalcq, 1988). However, T3 also facilitates an astrocytic fate (Johe et al., 1996). Thus, addition of T3 to neural stem cell cultures results in a mixed gliogenic population.

One important issue is the differences between rodent and human embryonic neural stem cells, regarding their response to cytokines and growth factors. For example, addition of PDGF and CNTF to human NSC cultures does not increase the number of neurons and astrocytes, respectively, as they do in their murine counterpart (Caldwell et al., 2001; Galli et al., 2000b). These findings highlight important differences between humans and rodents concerning the way exogenous cues regulate the function of neural stem cells.

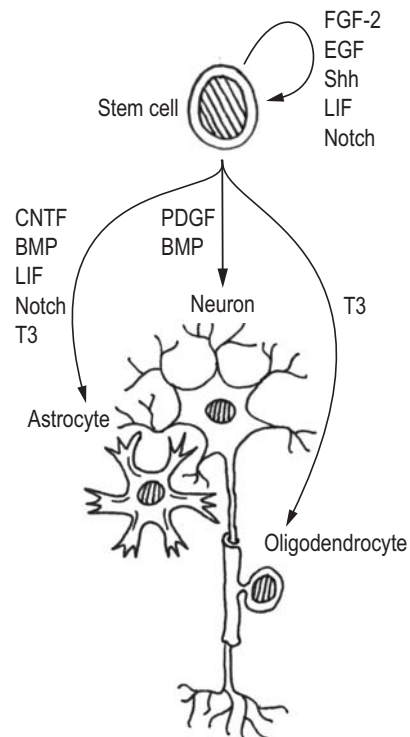


Figure 5. *Regulating factors of the CNS stem cell niche.*

Fibroblast growth factor (FGF)

Ligands and receptors

Fibroblast growth factors (FGFs) comprise a large family of proteins proposed to play important roles in the development and cellular homeostasis (Coumoul and Deng, 2003; Dono, 2003; Ford-Perriss et al., 2001). Almost all organs in the body express FGFs. The mammalian FGF family consists of 22 structurally related polypeptides known as FGF1 to FGF18 and FGF20 to FGF23. FGFs are monomeric, unlike most other growth factors, e.g. PDGFs, which are dimeric. Some FGFs are expressed intracellularly (FGF-14 and -11), whereas the extracellular matrix sequesters others (FGF-1 and -2). The timing of expression varies, for example FGF-3, -4, -8, -15, and -17 are expressed only during embryonic development.

Today, four FGF receptors (FGFR1-4) have been identified, but a preliminary additional FGF receptor, FGFR5, is under characterization, (Kim et al., 2001; Sleeman et al., 2001). The complete activation of FGF receptor signaling is dependent on the cell surface-bound heparan sulfate proteoglycans (HSPG) (Lin et al., 1999). HSPGs bind extracellular FGF ligands with high affinity and present them to the FGF receptors, generating dimerization of the receptors, as well as receptor-ligand complex stabilization (Figure 7) (Ornitz, 2000; Szébenyi and Fallon, 1999).

FGF and CNS development

Members of the FGF family and their receptors have been implicated in embryonic growth and patterning and, in particular, CNS development (Dono, 2003). Ten of the twenty-two presently known FGFs are expressed in the developing CNS, along with the four FGF receptors (Walshe and Mason, 2000). FGF-2, -8, -15, and -17 are the most important ligands during CNS development, and they are distributed differently throughout the CNS: FGF-2 and FGF-15 are generally expressed along the neural tube, whereas FGF-8 and -17 are tightly localized to specific regions of the developing brain and are only expressed during the early phases of proliferation and neurogenesis (Ford-Perriss et al., 2001; Xu et al., 2000). FGF-8 is expressed by the isthmus, an organizer that separates midbrain from hindbrain, during initial patterning of the brain vesicle (Crossley et al., 1996). Ectopic rostral FGF-8 expression results in transformation of forebrain into midbrain structure, indicating that FGF-8 determines midbrain identity (Crossley et al., 1996; Lee, 1997).

FGF-2 stimulates division of cortical multipotent stem cells and may also act on postmitotic neurons to promote differentiation and survival (Ghosh and Greenberg, 1995; Qian et al., 1997). It is present in the embryonic cortex

as early as E9 (Giordano et al., 1992; Powell et al., 1991) and throughout postnatal life (Gonzalez et al., 1990; Kuzis et al., 1995; Powell et al., 1991; Weise et al., 1993). By mid to late stages of neurogenesis FGF-2 and its receptor, FGFR-1, expression are down-regulated (Raballo et al., 2000). *Fgf-2* knockout mice are viable, fertile and by gross examination they are phenotypically indistinguishable from *Fgf-2* wildtype littermates. The knockout animals, however, have a dramatic decrease in the number of cortical neurons (Dono et al., 1998; Raballo et al., 2000) and the density of neurons in the motor cortex is reduced (Ortega et al., 1998), possibly due to inability of the postmitotic neurons to migrate (Dono et al., 1998). In addition, inactivation of *Fgf-2* causes delayed skin wound healing (Ortega et al., 1998) and reduced blood pressure (Dono et al., 1998). Furthermore, when FGF-2 is delivered into the cerebral ventricles of rat embryos, both volume and total number of neurons greatly increase (Vaccarino et al., 1999). In adult animals stem cell proliferation is evoked upon FGF-2 or EGF administration (Kuhn et al., 1997).

All FGF receptors are expressed during CNS development. FGFR1 is widely expressed throughout the CNS, whereas FGFR2-4 expressions are restricted to specific regions (Coumoul and Deng, 2003). *Fgfr1*-deficient mouse embryos die during gastrulation (Yamaguchi et al., 1994). However, chimeric mice generated by injecting *Fgfr1*^{-/-} ES cells into blastocysts (Deng et al., 1997) display defects in neural tube formation, leading to spina bifida. Specific deletions in later CNS development show *Fgfr1*'s involvement in midbrain and hindbrain development (Trokovic et al., 2003) and in olfactory bulb formation (Hebert et al., 2003). *Fgfr3* mutant mice exhibit delay in the appearance of terminally differentiated oligodendrocytes, together with an increased GFAP (astrocyte marker) expression, suggesting a role for FGFR3 in the regulation of oligodendrocyte and astrocyte differentiation in the CNS (Oh et al., 2003; Pringle et al., 2003).

Platelet-derived growth factor (PDGF)

Ligands and receptors

Platelet-derived growth factor (PDGF) was discovered in the mid '70s (Kohler and Lipton, 1974; Ross et al., 1974; Westermark and Wasteson, 1976) as a mitogen for connective tissue. Subsequently, PDGF was shown to be an important regulator of embryo development (Hoch and Soriano, 2003) and cellular proliferation, migration and survival (Betsholtz et al., 2001; Heldin and Westermark, 1999). The PDGFs are synthesized by many different cell types of endothelial, epithelial and neural origin. These cell types also express the PDGF receptors (Betsholtz et al., 2001; Heldin and Westermark, 1999). The PDGF receptors and their ligands are often found to be expressed in separate but adjacent cell layers (Orr-Urtreger and Lonai, 1992), suggestive of paracrine stimulation.

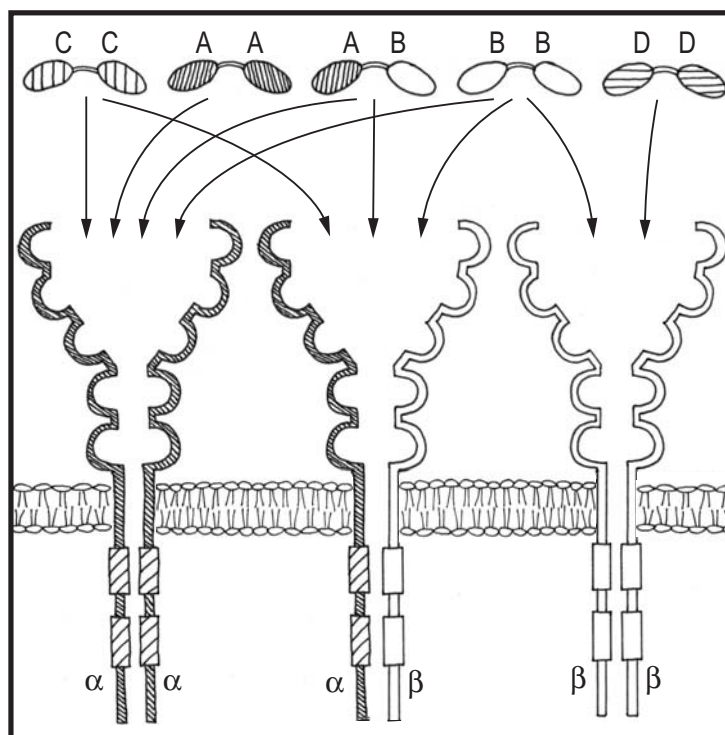


Figure 6. The PDGF family of ligands and receptors.

There are four members of the PDGF family (Figure 6): the classical PDGFs, PDGF-A and PDGF-B, which have been studied intensively for more than 20 years; and the recently discovered PDGF-C and PDGF-D (LaRochelle et al., 2001; Li et al., 2000). PDGF-C and -D differ from PDGF-A and -B in that they need to be proteolytically activated prior to receptor binding. All isoforms are active as homodimers (AA, BB, CC, and DD), whereas the classical PDGFs also can form a heterodimer (AB) (Betsholtz et al., 2001; Heldin and Westermark, 1999).

PDGFs bind to two receptor tyrosine kinases, PDGF α -receptor and PDGF β -receptor, which dimerize upon ligand binding. The dimeric receptors have different ligand-binding capacities: PDGFR- α has the broadest capacity and can bind all isoforms except the PDGF-DD homodimer, whereas PDGF- β only can bind PDGF-BB and -DD. The heterodimeric receptor PDGFR- β can bind PDGF-AB, -BB, and -CC, and there are indications that PDGF-DD also is able to bind to this receptor (Betsholtz et al., 2001).

PDGF and CNS development

PDGFs and their receptors are widely expressed in both embryonic and adult CNS, where PDGF was first reported to cause proliferation and differentiation of oligodendrocyte progenitor cells (Heldin et al., 1981; Noble et al., 1988; Raff et al., 1988). Since then, other possible roles of PDGF in CNS have been suggested, such as having neurotrophic effects (Smits et al., 1991), being involved in neuroprotection (Pietz et al., 1996), promoting neuronal differentiation (Erlandsson et al., 2001; Johe et al., 1996; Williams et al., 1997), and modulating synaptic transmission (Valenzuela et al., 1997). *In vivo*, *Pdgf-a* mRNA expression can be seen in the early developing mouse embryo, already at the blastocyst stage (Mercola et al., 1990). At E12 it is expressed in neurons in the spinal cord and dorsal ganglia, and from E15 expression is seen in most adult neurons (Fruttiger et al., 1999). PDGF-B can be detected in neurons in several CNS regions of the embryo and the adult. The olfactory system has the strongest and earliest expression of PDGF-B protein expression (Sasahara et al., 1992). *Pdgf-c* mRNA is mainly expressed in embryonic CNS, from E11, primarily in progenitor cells of the developing spinal cord, cerebellum, and the cerebral cortex ventricular zone (Aase et al., 2002; Ding et al., 2000; Hamada et al., 2002). The strongest expression of *Pdgf-d* mRNA is seen in motoneurons of the adult spinal cord, whereas during CNS development PDGF-D expression can be detected in thalamus and in the floor plate (Hamada et al., 2002).

PDGF β -receptors have predominantly been identified on glial precursors in various regions of the rat and mouse CNS, from E15-19 to postnatal life (Pringle et al., 1992; Yeh et al., 1993). This receptor is one of the most

commonly used markers for oligodendrocyte precursors. As myelination begins, PDGFR- α expression declines (Butt et al., 1997). In addition, radial glial cells in the E8.5 mouse embryo (Andrae et al., 2001) and E14 rat neural stem cell cultures (Forsberg-Nilsson et al., 1998) express PDGFR- α . Postnatally, α -receptors are also expressed by neurons (Oumesmar et al., 1997). The PDGF β -receptor is expressed in many neuronal cell types in the rat CNS (Smits et al., 1991), but not in CNS stem cells (Forsberg-Nilsson et al., 1998).

PDGF knockouts

Phenotypic analyses of gene knockouts in mice have greatly contributed to the understanding of PDGF's physiological functions. The mutant phenotypes of the different PDGF isoforms and receptors differ a lot, from embryonic lethality to adult viability.

Both *Pdgf-a* and *Pdgfr-* gene inactivation are lethal, but there are substantial phenotype differences between the knockout mouse strains. *Pdgf-a* null mice either die before E10, growth retarded, or survive up to six weeks after birth (Bostrom et al., 1996). The latter are suffering from a broad range of defects in several tissues, e.g. lack of lung alveolar smooth muscle cells, leading to lung emphysema (Bostrom et al., 1996; Lindahl et al., 1997), and oligodendrocyte deficiency (Fruttiger et al., 1999). The oligodendrocyte defect results in a severe reduction in the number of myelinated nerve fibers. Consequently, *Pdgf-a* null mice that survive postnatally develop a tremor phenotype (Fruttiger et al., 1999), similar to mutants with defects in key components of myelin. The *Pdgfr-* knockout phenotype is more severe than that of the *Pdgf-a* knockout, probably because this receptor also binds the PDGF-B and PDGF-C ligands. Mice die between E8-E16 with defects such as cleft face, spina bifida, and skeletal and vascular defects (Soriano, 1994; Soriano, 1997). These defects are very similar to the *Patch* mouse mutant, a naturally occurring mutant that carries a large genomic deletion encompassing *Pdgfr-* (Stephenson et al., 1991). Interestingly, *Pdgf-c* mutants also display cleft palate and spina bifida, similar to the *Pdgfr-* knockouts. In addition, *Pdgf-a/Pdgf-c* double knockout mice resemble phenotypes associated with *Pdgfr-* (C. Betsholtz, personal communication).

Inactivation of the genes for *Pdgf-b* (Leveen et al., 1994) or *Pdgfr-!* (Soriano, 1994) in mice gives similar phenotypes. The mice die during late gestation from cardiovascular, hematological and renal defects. *Pdgf-b* has also been ablated specifically in postmitotic neurons of transgenic mice (Enge et al., 2003). These mice survived to adulthood without apparent defects. Neither did the knockout affect the astroglial and angiogenic response to injury of the brain. Thus, the role of neuron-derived PDGF-B remains obscure.

FGF and PDGF receptor signaling

Receptor tyrosine kinases

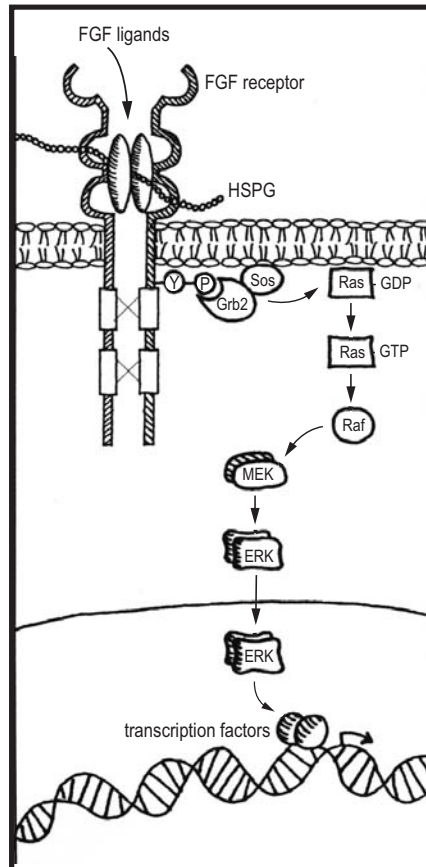
The receptors for both FGF and PDGF are receptor tyrosine kinases (RTKs), a subfamily of protein-tyrosine kinases. Other members of the RTK family are the receptors for e.g. insulin, EGF, and nerve growth factor (NGF). RTKs play important roles in the control of cell cycle progression, cell survival, migration, proliferation and differentiation. RTK signaling is tightly regulated in order to mediate normal cellular physiological responses (Schlessinger, 2000).

RTKs are membrane-spanning cell surface receptors, composed of an extracellular ligand binding domain, that is connected to the cytoplasmic domain by a single transmembrane helix. The cytoplasmic domain contains a conserved protein tyrosine kinase core. Most RTKs are monomeric in the absence of ligand, but when the ligand binds, the monomeric receptors dimerize. Upon dimerization, specific tyrosine residues in the cytoplasmic portion become autophosphorylated (Ullrich and Schlessinger, 1990), which stimulates the intrinsic kinase activity of the receptor or generates recruitment sites for downstream signaling proteins.

Intracellular signaling molecules that bind to the activated receptor contain phosphotyrosine-recognition domains, such as the Src homology 2 and 3 (SH2 and SH3) domains or the phosphotyrosine-binding (PTB) domain (Pawson, 1995). Adaptor proteins, e.g. Grb2, Shc, and Crk, are devoid of enzymatic activity and they utilize their SH2 and SH3 domains (recognizing proline-rich sequences) to mediate interactions that link different proteins involved in signal transduction. For example, Grb2 interacts with activated RTKs via its SH2 domain and recruits Sos, a guanine nucleotide releasing factor, thereby linking the receptor to the Ras/mitogen-activated protein (MAP) kinase signaling pathway (Pawson, 1995; Schlessinger, 1994; Schlessinger, 2000). Other SH2 domain proteins that couple RTKs to intracellular signaling pathways have enzymatic activity, e.g. the SHP-2 tyrosine phosphatase. Upon FGF stimulation, SHP-2 binds to the FGF receptor via the docking protein FGF receptor substrate 2 (FRS2) (Hadari et al., 1998; Kouhara et al., 1997), and recruits the Grb2/Sos complex.

Ras/MAP kinase signaling pathway

The Ras/MAP kinase pathway is prominent among mitogenic signaling pathways, and it is also implicated in migration and differentiation.



Sos leaves the complex with Grb2 and translocates to the membrane, where it stimulates the exchange of GDP for GTP on the small G protein, Ras. Activated Ras binds to the serine/threonine kinase Raf-1, which activates MEK (MAP kinase/ERK kinase). MEK subsequently phosphorylates the MAP kinases extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2) on tyrosine and threonine residues, leading to activation. Activated ERKs phosphorylates a variety of cytoplasmic and membrane-linked substrates. In addition, ERK is rapidly translocated into the nucleus where it phosphorylates and activates transcription factors (Hunter, 2000; Karin and Hunter, 1995).

Figure 7. The Ras/MAP kinase signaling pathway. The diverse set of SH2-containing proteins that couple FGF- and PDGF-receptors to the Ras/MAP kinase pathway are not included.

Cancer

Transforming activity of PDGF

Although PDGFs play important roles in normal development, they also contribute to a variety of diseases, including cardiovascular and fibrotic diseases, and cancers.

In the early '80s, the transforming *v-sis* oncogene of Simian Sarcoma Virus (SSV) was shown to be the viral homolog of the cellular *Pdgf-b* gene (Doolittle, 1983; Waterfield, 1983). Since then, a lot of attention has been given to the potential role of PDGF in autocrine transformation. Specific mutations of *Pdgf* and its receptors have been described in human cancers,

leading to deregulated expression of the growth factor as well as increased activity of the receptor (Heldin and Westermark, 1999).

In many different human tumors PDGF ligands and receptors are co-expressed, which potentially enables autocrine and/or paracrine stimulation. The involvement of PDGF in the etiology of tumors was demonstrated by SSV injection into marmoset brain, resulting in sarcomas and brain tumors (Deinhardt, 1980). Furthermore, retroviral induction of *Pdgf-b* into mice brains gave rise to fibrosarcomas (Pech et al., 1989) and tumors with characteristics of glioblastoma multiforme (Uhrbom et al., 1998). Amplification of *Pdgfr-* has also been seen in some glioblastomas (Fleming et al., 1992; Hermanson et al., 1996; Kumabe et al., 1992), where progression of the tumor into higher grades acquired increased *Pdgfr-* expression levels (Hermanson et al., 1996). In addition, PDGF-C and PDGF-D are highly expressed in several brain tumor specimens, indicating a role in glioblastoma development (Lokker et al., 2002). Other types of brain tumors besides gliomas also express PDGF and PDGFR, such as medulloblastomas (Andrae et al., 2002; Gilbertson and Clifford, 2003; Smits et al., 1996; Whelan et al., 1989).

PDGF transgenic mice

Transgenic mouse models have given the opportunity to examine the consequences of temporal and/or spatial *Pdgf* overexpression. When mice with a deregulated expression of *Pdgf* are generated, one key question is if these mice are more prone to develop tumors compared to wildtype mice. Mice with specific *Pdgf-a* and *Pdgf-b* overexpression in the oligodendrocyte lineage exhibit hyperproliferation of oligodendrocyte progenitors, but no tumor formation (Calver et al., 1998; Forsberg-Nilsson et al., 2003). In addition, recent results suggest that transgenic overexpression of *Pdgf-a* increases the number of oligodendrocyte progenitor cells upon toxin-induced demyelination (Woodruff et al., 2004). These data further support the role of PDGFs as mitogens for glial cell types. Using the retroviral RCAS/tv-a system, Dai *et al* achieved postnatal *Pdgf-b* overexpression in nestin-expressing or GFAP-expressing cells (Dai et al., 2001). These mice developed low-grade oligodendrogliomas and oligoastrocytomas, respectively, indicating that autocrine PDGF stimulation alone, without additional mutations, might be sufficient for gliomagenesis.

Stem cells and brain tumors

Neural stem/progenitor cells may be implicated in cancer by two means; one as being the cause of tumorigenesis and another as a possible anti-tumorigenic actor.

Primitive neuroectodermal tumors (PNETs) are a heterogenous group of highly malignant tumors that affects children. Both neurons and glial cells have been detected in these tumors (Fung et al., 1995), as well as expression of the neural stem cell marker nestin (Dahlstrand et al., 1992; Valtz et al., 1991). All together, these data indicates that transformation of neural stem/progenitor cells might be the first step in the development of PNETs. Nestin is also expressed in gliomas, and in these tumors the level of nestin expression has been shown to correlate with the grade of malignancy (Dahlstrand et al., 1992).

Neural stem cells are not only a possible origin of CNS tumors, but may also constitute a treatment instrument. Neural stem/progenitor cells possess features that are interesting for the treatment of many pathological conditions: they are highly migratory and show affinity for pathological areas (Arvidsson et al., 2002; Nakatomi et al., 2002). Furthermore, they are multipotent, allowing them to engraft and replace damaged tissue in the CNS. The lethal nature of gliomas is in part due to the migration of glioma cells into the normal brain parenchyma. It was shown a few years ago that neural stem cells have the ability to target and surround the invading tumor border (Aboody et al., 2000). In addition, neural stem cells containing the apoptotic ligand TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) was shown to induce apoptosis of human glioblastoma xenografts and inhibit tumor growth (Ehtesham et al., 2002). These results suggest a potential of neural stem cells as therapeutically effective delivery vehicles for the treatment of intracranial glioma.

Is a stem cell really a stem cell?

Neural stem cell culture systems

Neural stem/progenitor cells can be propagated *in vitro* using two different protocols, either as free-floating aggregates, called neurospheres, or as monolayer cultures grown on substrates. The cell culture system used in this thesis is based on monolayer cultures of neural stem/progenitor cells from rat embryonal day 14.5 cerebral cortex. Cells are cultured on poly-L-ornithine- and fibronectin-coated tissue culture dishes in serum-free medium supplemented with FGF-2 (Johe et al., 1996). Upon withdrawal of the mitogen,

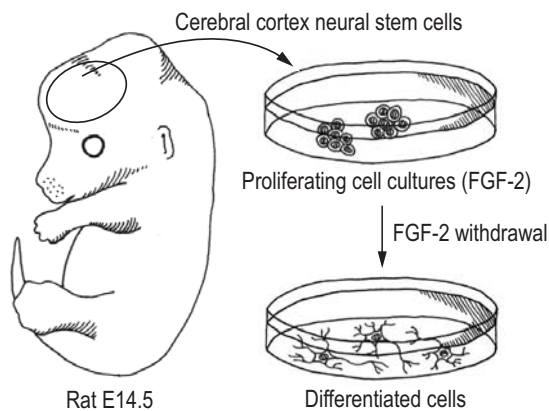


Figure 8. *Monolayer cultures of rat cortical neural stem/progenitor cells.*

neural stem cells spontaneously start to differentiate into neurons, astrocytes, and oligodendrocytes.

Clonal analyses have demonstrated that neurosphere formation begins with the proliferation of a single neural stem cell, followed by symmetric and asymmetric cell divisions to self-renew neural stem cells and to produce mitotically active progenitor cells. It is therefore very likely that the

majority of the cells in neurospheres and adherent cultures are progenitors, whereas cells with the ability to regenerate a clone (Reynolds and Weiss, 1996) constitute only a very small proportion (Cai et al., 2002; Davis and Temple, 1994; Kalyani et al., 1997; Tropepe et al., 1999). Because of the heterogeneity of cell cultures, one should exert caution when interpreting data from these cell systems. However, by using FACS analysis successful purifications of neural stem cells have been performed, generating more homogenous stem cell cultures, (Maric et al., 2003; Rietze et al., 2001; Uchida et al., 2000).

An additional concern is whether the cultured cells retain properties of *in vivo* neural stem/progenitor cells. Recent studies have compared the expression of positional identity genes in neurospheres with neural tube regions that they were derived from. Two studies demonstrated that the positional identity of origin of the stem/progenitor cells was preserved in culture (Hitoshi et al., 2002; Parmar et al., 2002), whereas a third study showed a partial loss of positional identity upon neurosphere formation (Santa-Olalla et al., 2003).

The two cell culture systems mentioned have several advantages and disadvantages. By growing cells in a three-dimensional manner larger cell numbers can be obtained, and neurosphere cultures imitate the *in vivo* situation more than monolayer cultures do. On the other hand, when studying the effect of different exogenously added factors, cells grown as monolayers have the same accessibility to the factors. In this respect, when working with neurosphere cultures, it is very important to keep the neurospheres small. Another advantage of using monolayer cultures compared to neurospheres is the ability to follow the morphology of the cells during the time of culture. However, the identification of a specific cell type is only in part determined by the morphology of the studied cell, but mostly relies on the recognition of cell type-specific molecular markers.

Cell-specific molecular markers

The identity of embryonic and adult neural stem cells remains controversial. Lack of exclusive neural stem cell markers makes it difficult to demonstrate long-term self-renewal and multipotency *in vivo*. Furthermore, the identity of NSCs could change spatially (according to neural tube position) and temporally (according to developmental age). Neural stem and progenitor cell identity is in part defined by their expression of different sets of transcription factors, such as Sox1 and Sox2 (Pevny et al., 1998; Sasai, 2001), and proneural basic helix-loop-helix (bHLH) transcription factors (e.g. neurogenins and Mash1) (Casarosa et al., 1999; Ma et al., 1998; Torii et al., 1999). Recently discovered intrinsic signals important for neural stem cell self-renewal and/or proliferation are Bmi-1 and nucleostemin. Bmi-1 is a polycomb family transcription repressor that seems to be solely required for CNS stem cell self-renewal, and not for progenitor proliferation (Molofsky et al., 2003), whereas the nuclear protein nucleostemin is required for both self-renewal and proliferation (Tsai and McKay, 2002).

Today, the most common markers used for neural stem cells are the intermediate filament nestin (Lendahl et al., 1990), the RNA-binding protein musashi (Sakakibara et al., 2002; Yagita et al., 2002), and the transcription factors Sox1 and Sox2 (Table I). Nestin, however, is not exclusively expressed in neural stem cells, but also in radial glia (Dahlstrand et al., 1995; Tohyama et al., 1992), developing muscle cells (Sejersen and Lendahl, 1993) and in reactive astrocytes (Clarke et al., 1994). The nestin expression diminishes upon differentiation of NSCs, although some nestin can be detected in lineage-restricted progenitors (e.g. neuronal and glial progenitors). Today, a combination of Sox1, Sox2, nestin and musashi is probably the most appropriate choice for NSC identification, despite their overlapping pattern with sublineage precursors (Mayer-Proschel, Neuron, 1997; Kaneko, Dev Neurosci, 2001; Rao, PNAS, 1998). However, combinations of positive and negative markers (Cai et al., 2002) or, alternatively, new unique NSC markers are required to unequivocally identify NSCs.

As the pure neural stem cell gradually develops into a lineage-restricted progenitor cell, it coincidentally starts to express new proteins. This protein expression pattern changes during the entire differentiation process, until the mature cell type has formed. Both traditional and new molecular cell markers that can be used for the identification of the CNS stem cell niche are listed in Table I. There is also a lack of distinct cellular markers for more mature cells. For example, GFAP, which traditionally has been used as an astrocyte marker, is expressed also in some stem cell populations, ependymal cells, and in radial glial cells. To solve the problems with the overlapping protein expression pattern of neural stem and progenitor cells and their postmitotic progeny, we need to find new, more specific markers.

Table 1. *Examples of traditional and new cellular markers for the neural stem cell lineage. The listed markers are not always cell type-specific, but can be expressed in other cell types as well.*

Cell type	Markers
Neural stem cells	Sox1 and Sox2 - transcription factor (Pevny et al., 1998; Sasai, 2001) Nestin - class VI intermediate filament (Lendahl et al., 1990) Musashi - RNA-binding protein (Sakakibara et al., 2002; Yagita et al., 2002) Bmi-1 - polycomb family transcription repressor (Molofsky et al., 2003) Nucleostemin - nuclear protein (Tsai and McKay, 2002) Vimentin - class III intermediate filament (Houle and Fedoroff, 1983)
Immature neurons	Hu - human autoantibody (Marusich and Weston, 1992) MAP2 - Microtubule-associated protein 2 (Garner et al., 1988; Matus, 1988) ! -III tubulin - cytoskeletal protein (Caccamo et al., 1989)
Mature neurons	NF - Neurofilament (Huneeus and Davison, 1970) NeuN - neuronal nuclear antigen A60 (Mullen et al., 1992) NSE - neuron-specific enolase (Sensenbrenner et al., 1997) Synaptophysin - presynaptic vesicle membrane polypeptide (Wiedenmann and Franke, 1985) Tau - Microtubule-associated protein (Viereck et al., 1988) TH - Tyrosine hydroxylase, dopaminergic neurons (Pickel et al., 1975) GABA - γ -aminobutyric acid, neurotransmitter (Kisvarday et al., 1990)
Radial glial cells	Nestin - class VI intermediate filament (Lendahl et al., 1990) Vimentin - class III intermediate filament (Houle and Fedoroff, 1983) GLAST - glutamate transporter (Shibata et al., 1997) BLBP - brain lipid-binding protein (Feng et al., 1994) RC1 and RC2 - (Edwards et al., 1990; Misson et al., 1988)
Immature astrocytes and oligodendrocytes	PDGFR- β - expressed in oligodendrocyte-type2-astrocyte cells (Ellison and de Vellis, 1994) A2B5 - ganglioside (Hirano and Goldman, 1988) NG2 - chondroitin sulphate proteoglycan (Nishiyama et al., 1999) Olig 1 - bHLH transcription factor (Zhou et al., 2000) Sox10 - SRY box containing transcription factor (Kuhlbrodt et al., 1998) Nkx2.2 - homeodomain transcription factor (Qi et al., 2001) Ngn3 - neurogenin, bHLH transcription factor (Liu et al., 2002)
Mature astrocytes	GFAP - glial fibrillary acidic protein intermediate filament (Eng et al., 2000) CD44 - hyaluronate receptor (Alfei et al., 1999; Moretto et al., 1993) S100- β - calcium binding protein (Zimmer et al., 1995) GLAST - astrocyte-specific glutamate transporter (Shibata et al., 1997)
Mature oligodendrocytes	O4 - cell surface antigen (Sommer and Schachner, 1981) GalC - galactocerebroside (Raff et al., 1978) CNPase - 2', 3'-cyclic nucleotide 3'-phosphodiesterase (Trapp et al., 1988) MAG - myelin-associated glycoprotein (Sternberger et al., 1979) MBP - myelin basic protein (Hartman et al., 1982) PLP - myelin proteolipid protein (Hartman et al., 1982)

The microarray analysis technology enables an efficient screening of the total gene expression pattern of a cell population. With this experimental approach, classes of genes have been identified that are involved in neural stem cell proliferation or in the formation of neurons and glia (Geschwind et al., 2001; Karsten et al., 2003; Luo et al., 2002). Microarray analysis has also been used for trying to identify genes expressed in different types of stem cells, so called "stemness" genes (Ivanova et al., 2002; Ramalho-Santos et al., 2002; Terskikh et al., 2001). The accurate comparisons of the results from these studies are, however, complicated by the various cell culture systems that have been used. Nevertheless, this tool can hopefully provide us with increased knowledge of regulated genes in the discussed individual cell populations, generating a complete map of the neural stem cell lineage and new specific molecular cell markers.

Present investigation

The general aim of neural stem cell research is to acquire an increased knowledge of central nervous system development and to enable the use of stem cells in the therapy of CNS diseases and damages. The focus of this thesis was to investigate mechanisms of neural stem cell proliferation and differentiation and the roles of platelet-derived growth factor (PDGF) in these events. The aim of the specific papers in this thesis was to:

- I Clarify the role of PDGF in neuronal differentiation
- II Investigate the importance of ERK signaling during initial neuronal differentiation
- III Identify genes involved in neural stem cell differentiation and to monitor genes regulated by PDGF
- IV Study the effects of PDGF-B overexpression in neural stem cells of transgenic mice

Results and discussion

Immature neurons from CNS stem cells proliferate in response to PDGF (paper I)

The identification of external signals involved in the regulation of neural stem cell proliferation and differentiation is fundamental to the understanding of CNS development. PDGF has been suggested to play a role in neuronal differentiation of neural stem cells (Johe et al., 1996; Williams et al., 1997) but the underlying mechanism of this action has not been known. We show in this study that PDGF acts as a mitogen and a survival factor for immature neurons, and thereby support neuronal differentiation. In addition,

we delineate the expression and activation pattern of PDGF receptors on cultured CNS stem cells.

Western blot analysis was used to characterize PDGF receptor expression and phosphorylation in proliferating and differentiating neural stem cell cultures. We could detect PDGF α -receptors in stem cell cultures from rat E14.5 cerebral cortex proliferating in the presence of FGF-2. Upon withdrawal of the mitogen, stem cells spontaneously start to differentiate into neurons, astrocytes and oligodendrocytes. The PDGFR- α expression was stable during 6 days of differentiation, but no phosphorylation of the receptor was detected. Addition of PDGF-AA at the time of FGF-2 withdrawal, however, induced a strong receptor phosphorylation. The PDGF β -receptor was not expressed in proliferating cells, but was up-regulated during differentiation. In contrast to PDGFR- α , the β -receptor was phosphorylated in the absence of exogenous ligand. Thus, we suggest that the PDGF α -receptor is the predominant receptor isoform in neural stem cells. It is likely that the β -receptor up-regulation reflects the expression on neurons, as signaling through the PDGF β -receptor has been reported to be involved in neuronal survival and proliferation (Giacobini et al., 1993; Nikkhah et al., 1993).

PDGF stimulation of neural stem cells affects several cellular events. To demonstrate the mitogenic activity of PDGF-AA, we showed that PDGF-AA-treated cultures incorporate 5'-bromo-2'deoxyuridine (BrdU) and have a four-fold increase in total cell number compared to untreated cells. After a few days of differentiation, neural stem cells grown in the absence of PDGF express the neuronal marker microtubule-associated protein 2 (MAP2) and exhibit a neuronal morphology with long extended processes. PDGF-treated cell cultures also express MAP2, but contrary to untreated cultures these cells extend no processes and have a round-shaped immature morphology. To investigate whether the round, immature cells were proliferating, we double-stained the cultures with antibodies to BrdU and MAP2 and could detect a population of dividing neuronal cells. These results clearly demonstrate that PDGF acts as a mitogen for immature neurons derived from multipotent stem cells. PDGF-AA also exerts a cell survival effect on neural stem cells. TUNEL staining showed that the number of apoptotic cells was reduced by half after four days in the presence of PDGF-AA compared to untreated cultures. Furthermore, the PI-3-kinase/Akt pathway, which mediates cell survival signaling, was activated in PDGF-AA-treated cultures but not in control cultures.

Our results suggest that PDGF-AA does not function as an instructive agent for neuronal differentiation, but rather acts as a proliferation and survival factor for a population of immature neurons.

ERK signaling is uncoupled from initial differentiation of CNS stem cells to neurons (paper II)

In paper I, we investigated the role of PDGF-AA on neural stem cell differentiation and showed that it supports neuronal progenitor survival and proliferation. Which signaling pathways are then activated upon PDGF-AA-treatment in addition to the PI-3-K/Akt signaling pathway? We next aimed at examining the activated signaling cascades in both proliferating (FGF-2-treated) and differentiating (+/- PDGF-AA-treatment) primary neural stem cell cultures.

Signaling via the Ras/MAP kinase pathway is central in many cellular events. In neuronal cell lines, the duration of the MAP kinase ERK (extracellular signal-regulated protein kinase) phosphorylation has been suggested to play a role for cell fate decision. In PC12 pheochromocytoma cells, a prolonged ERK activation is sufficient for differentiation whereas a transient ERK activation promotes proliferation (Cowley et al., 1994; Fukuda et al., 1995; Qui and Green, 1992; Traverse et al., 1992). However, prolonged ERK activation is not necessary for differentiation of the hippocampal cell line, H19-7 (Kuo et al., 1996; Kuo et al., 1997). In addition, chicken root ganglia can differentiate in the absence of ERK signaling (Klinz et al., 1996). Thus, previous studies present conflicting results with regard to the role of ERK in neuronal differentiation. We show in this paper that both FGF-2 and PDGF-AA phosphorylate ERK in a sustained manner, although FGF-2 is a more potent activator than PDGF-AA. Our data indicate that ERK activation is prolonged in proliferating primary neural stem cell cultures, but not in differentiating cultures.

What happens to neural stem cells when the Ras/MAP kinase pathway is shut off? We used a small molecule inhibitor of the upstream activator of ERK, MEK, to study the role of ERK during early stem cell differentiation. When neuronal precursor cells become postmitotic, the expression of the proneural bHLH transcription factor MASH1 ceases. Therefore, we used MASH1 downregulation as a marker of initial differentiation. Neural stem cell cultures that were treated with the MEK inhibitor downregulated MASH1 protein expression one day after FGF-2 withdrawal, as did control cells not exposed to the inhibitor. This indicated that the differentiation program had started without active ERK signaling. Furthermore, we showed that MAP2-positive cells with a neuronal morphology could form in the absence of active ERK, at least during the first days of differentiation.

In contrast to untreated cultures, a prolonged MASH1 expression was observed in cells differentiating in the presence of PDGF-AA. We observed that the total cell number in cultures treated with a combination of the MEK inhibitor and PDGF-AA was lower than in cells treated with PDGF-AA alone. This is in line with the data presented in paper I and possibly indicates that the PDGF-AA-induced proliferation and/or survival of MAP2-positive

cells are dependent on Ras/MAP kinase signaling. To summarize, it seems that Ras/ERK signaling is not important for the earliest steps of neuronal differentiation, but probably for neural stem/progenitor cell proliferation.

Analysis of gene expression in neural stem cells during proliferation and differentiation (paper III)

An issue in neural stem cell research is the lack of cell-specific markers and, consequently, the difficulties to identify cells in the neural stem cell lineage. A promising tool to use in the search for new markers is the microarray technology, which enables simultaneous assessment of thousands of genes in a certain cell population. We have used a 15K embryonic mouse microarray to investigate the gene expression profile of neural stem cell monolayer cultures. The aim was to identify genes associated with the stem cell state as well as genes expressed during early and late stages of differentiation.

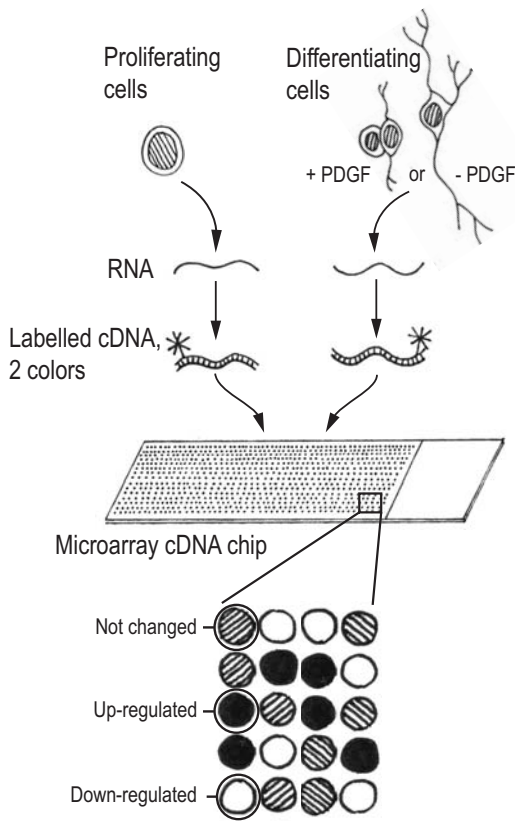


Figure 9. *The microarray technology.*

well as genes expressed during early and late stages of differentiation. In addition, we wanted to characterize genes specifically regulated by PDGF-AA.

Proliferating rat neural stem cell cultures (FGF-2-treated cells) were compared to cells that had differentiated for 12 hours or 6 days, in the absence or presence of PDGF-AA. We received a comprehensive list of down-regulated and up-regulated genes, i.e. genes that were enriched in proliferating cells and differentiating cells, respectively (Figure 9). We chose a number of genes known to be involved in neural stem cell biology, as well as a set of unknown genes and verified their expression profile by quantitative real-time RT-PCR. By this method, we could follow the detailed

kinetics of the gene expression and we could also verify that the rat cDNA had been properly hybridized to the mouse cDNA chip.

As expected, proliferating cell cultures expressed a higher amount of genes that are involved in cell cycle and DNA replication (44 genes), compared to differentiated cultures (5 genes). On the other hand, genes of many structural proteins, such as the neural lineage proteins synaptotagmin, tau, and myelin proteolipid protein, were enriched in differentiating cells (37 compared to 5 in proliferating cells).

One aim was to identify novel genes important for the initial steps of differentiation of neural stem cells. In a first attempt to investigate genes that may be early regulators of differentiation, we chose three ESTs that were up-regulated after 12 hrs differentiation. These were *tmstde*, *pink1* homologue, and a kinase domain-containing gene. The functions of these genes are unknown, but there are indications of their involvement in tumorigenesis and neural development. To investigate whether these three novel genes are involved in neural differentiation a detailed characterization is needed.

In paper I, we showed that PDGF is not an instructing agent for neuronal differentiation. Rather, PDGF seems to retain neurons in an immature progenitor state, expressing both markers for the neuronal lineage and cell proliferation. We therefore wanted to compare the genetic profile of PDGF-treated cells to that of proliferating and untreated differentiated cells. Our data did not single out individual genes to be specifically regulated by PDGF, compared to differentiating cells. According to the gene expression pattern, PDGF-treated neural stem cells seem to be an intermediate between proliferating cells and differentiating cells. These results support the idea that PDGF causes expansion of progenitor cells rather than acting solely as an instructing agent for neuronal differentiation.

Overexpression of PDGF-B in neural stem cells leads to increased apoptosis in the developing striatum (paper IV)

In this study, we wanted to investigate the consequence of deregulated *Pdgf-b* in nestin-expressing neural stem/progenitor cells *in vivo*. As shown in paper I, PDGF affects neural stem/progenitor cells by acting as a mitogen for neuronal progenitor cells *in vitro*. PDGF also has transforming capacity and is implicated in a number of brain tumors. We generated mice that overexpress *Pdgf-b* in the developing CNS, with the aim to answer several questions: Does a deregulated *Pdgf-b* expression change the proportion of cells differentiating into the various neural cell lineages? Do transgenic brains have a more immature cell phenotype compared to their wildtype littermates? Are these mice prone to develop brain tumors? In this paper we describe the establishment and initial characterization of transgenic mice overexpressing *Pdgf-b* in the developing neuroepithelium (Figure 10).

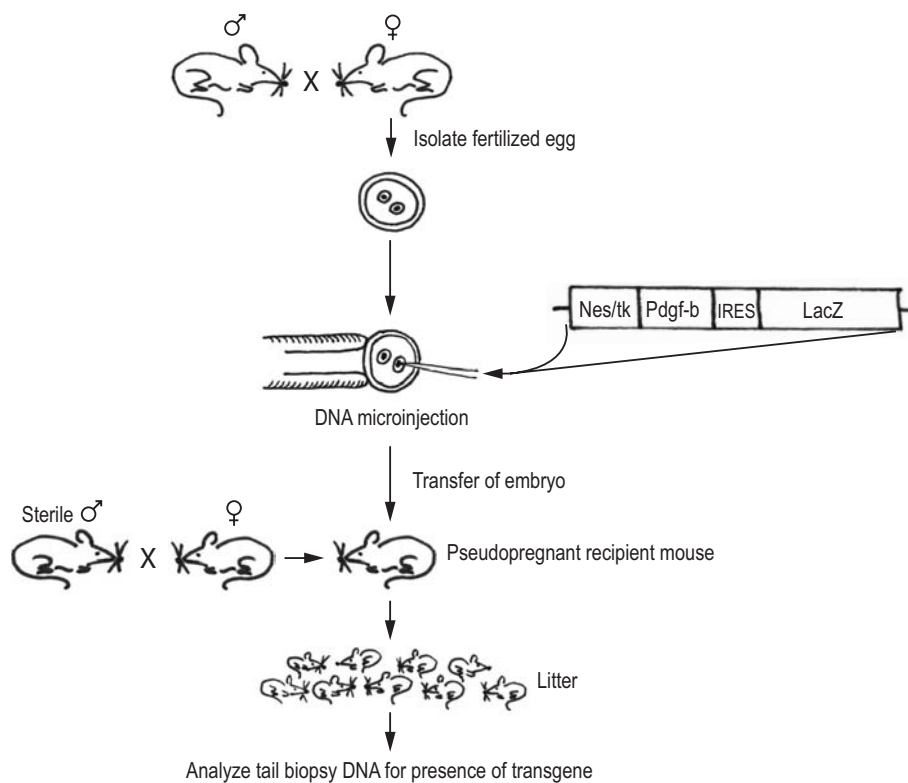


Figure 10. *Generation of transgenic mice.*

We used a transgenic construct consisting of a human *Pdgf-b* cDNA fragment coupled to a lacZ reporter gene. As a regulatory element driving the transgenic expression to the developing CNS, we used the nestin enhancer (Lothian and Lendahl, 1997). To test the functionality of the construct prior to microinjection, we transfected it into rat neural stem/progenitor monolayer cultures. Transfected cells exhibited β -galactosidase activity, thus showing that the transgene could be properly expressed.

Transgenic mice were generated and three lines (310, 312 and 319) from individual founders were analyzed for β -galactosidase activity and *Pdgf-b* mRNA expression. The whole-mount β -galactosidase assay on embryonic day 10.5 (E10.5) embryos showed transgene expression along the whole neural tube. At this embryonic stage, neural stem/progenitor cells reside throughout the neural tube, both in cranial and more caudal regions. One of the lines, 310, exhibited a stronger transgene expression than the other two. Two days later, at E12.5, the expression in line 312 and 319 was more restricted to certain areas, such as neopallial cortex and mesencephalon, and no transgene expression could be observed in the spinal cord. Line 310, how-

ever, still showed strong β -galactosidase activity in the spinal cord. Transcripts of the *Pdgf-b* gene were then detected by *in situ* hybridization on E14.5 brain sections. *Pdgf-b* mRNA expression was restricted to the germinal zones lining the ventricular system, thus corresponding to the endogenous nestin expression at this age (Dahlstrand et al., 1995).

Immunohistochemical stainings for nestin, Ki67 (proliferating cells), and MAP2 (neurons) were performed on E14.5 brain sections. We were not able to detect any differences in expression levels of these markers between transgenic and wildtype mice. These results indicate that elevated *Pdgf-b* expression does not induce increased proliferation of neural stem/progenitor or neuronal cells, at least not at this developmental stage. A deregulated *Pdgf-b* expression has in previous studies been shown to cause hyperplasia in transgenic mice (Forsberg-Nilsson et al., 2003) and tumors in retroviral insertion models *in vivo* (Dai et al., 2001; Uhrbom et al., 1998). One explanation of the different phenotypes could be that the ability of PDGF to cause substantial increase in cell number might be dose-dependent, and that there is a need for higher levels than those expressed in this mouse model. At this stage of analysis, we do not know to what levels the transgenic *Pdgf-b* mRNA is translated to protein.

We could detect an effect on cell survival in transgenic brains. TUNEL staining on E14.5 brain sections revealed increased apoptosis in the lateral ganglionic eminence (LGE) of the developing striatum. Apoptosis plays a role during normal brain development, but the quantity and significance of cell death within the germinal matrices is currently under debate (Brazel et al., 2003). Several studies demonstrate that the elimination of progenitors is essential for regulating progenitor numbers (Kuida et al., 1998; Thomaidou et al., 1997). Thus, the increased apoptosis in transgenic brains may be a secondary effect of *Pdgf-b* overexpression. It is possible that the transgenic mice actually have an increased number of progenitor cells due to the deregulated *Pdgf-b*, but that the endogenous safe guarding system can induce cell death of these cells. Alternatively, progenitor cells may die due to lack of trophic support (other than PDGF). Another possibility is that the excess of PDGF has disturbed the extensive migration of neuronal progenitors that occurs in the LGE at this embryonic age. In a recent report, deregulated *Pdgf-b* expression in the developing cerebellum was shown to disrupt the cellular migration during the cerebellar midline fusion process (Andrae et al., in press). However, the mechanisms behind the increased apoptosis in the LGE of the nestin-*Pdgf-b* transgenic mice remain to be elucidated.

Future perspectives

During the last decade CNS stem cell research has contributed to increased understanding of normal CNS development as well as CNS pathologies. However, future efforts must continue to address the identity of stem cells and their progeny, the factors that regulate the stem cell fate, and the characteristics of both endogenous and transplanted stem cells *in vivo*.

The focus of this thesis has been the underlying mechanisms of neural stem/progenitor cell proliferation and differentiation. The role of the growth factor PDGF in these cellular events has been investigated. To summarize, we have found that PDGF promotes survival and proliferation of rat neuronal progenitor cells and that the Ras/ERK signaling pathway probably mediates the mitogenic activity of PDGF. In contrast, neuronal differentiation is not dependent on the Ras/MAP kinase pathway. Studying the genetic profile of PDGF-treated cells, we came to the conclusion that these cells are an intermediate between proliferating neural stem cells and their differentiated progeny.

To study the effect of PDGF on neural progenitor cells *in vivo*, we generated transgenic mice that overexpress *Pdgf-b* in the developing CNS using the nestin enhancer. An initial characterization of embryonic day 14.5 transgenic brains was made. Surprisingly, we observed neither signs of increased neuronal progenitor cell proliferation nor more immature neuronal cell phenotypes in the neuroepithelium. Instead, increased apoptosis was detected in the developing striatum, probably as a result of deregulated PDGF signaling. How does this embryonic striatal apoptosis affect the brain of postnatal mice? We will next analyze the cellular composition and brain morphology of both neonatal and adult transgenic brains. In addition to the cellular markers that we have used so far, we will stain brain sections with astrocyte, oligodendrocyte, and radial glial cell markers. At present we have studied the *Pdgf-b* mRNA expression in transgenic brains and we will now investigate PDGF-B protein levels, by immunohistochemistry as well as proximity-dependent DNA ligation. The PDGF α - and β -receptor expression and phosphorylation patterns will also be examined.

Furthermore, we will investigate if the nestin-*Pdgf-b* transgenic mice are prone to develop tumors. Holland and colleagues retrovirally transduced *Pdgf-b* to nestin-expressing cells in transgenic mice (Dai et al., 2001). These mice developed low-grade oligodendrogliomas. So far, we have not observed any signs of malignancies in our transgenic lines. Possible explana-

tions for the different phenotypes can be that 1) Dai *et al* used a retroviral model mediating a postnatal *Pdgf-b* expression, contrary to our embryonic transgene expression; 2) the retroviral method may cause additional mutations depending on the insertion site of *Pdgf*, although this is less likely because the tumors developed early; 3) the transformation effect of *Pdgf* may be dose-dependent and our transgenes possibly express too low levels of *Pdgf-b* for a transformation event to occur.

Because development of tumors has been associated with several consecutive mutations we will introduce genetic alterations by breeding the nestin-*Pdgfb* mice to mice deficient in tumor suppressor genes. These mice will be analyzed for tumor development.

In our attempts to find new genes that are important for the early differentiation steps of neural stem cells, we will analyze novel genes from the microarray gene list. *In situ* hybridization on embryonic neural tube will be performed to investigate which cell types that express the novel genes. In addition, protein levels will be analyzed by immunohistochemistry after raising peptide antibodies against the uncharacterized proteins. We will also perform immunofluorescence stainings of neural stem cell cultures with antibodies against the novel proteins in combinations with other antibodies, such as nestin, Ki67, MAP2, GFAP, and O4. To investigate if the candidate genes can be linked to tumors, we will study protein expression in human brain tumor cell lines and patient material.

One way to investigate the functions of a certain gene is to suppress gene expression. We have designed short interference RNA (siRNA) constructs for transfection to neural stem cells. To study if the candidate genes are necessary for neuronal or glial differentiation we will stain siRNA-transfected cells with antibodies against the different cell lineages. If the candidate genes yield interesting results *in vitro*, we will generate transgenic mice to be able to analyze the *in vivo* function of the novel candidate proteins.

Populärvetenskaplig sammanfattning

Kroppens organ består av celler, som har olika funktioner beroende på vilket organ de tillhör. Exempelvis behöver hjärnan signalerande nervceller, medan vissa av bukspottskörtelns celler har till uppgift att producera insulin. Under fosterutvecklingen (den embryonala utvecklingen) bildas celltyperna från omogna celler, så kallade stamceller. Stamceller kan, förutom att bilda nya mogna celltyper, dela sig och göra kopior av sig själva (Figur 11). De här egenskaperna har gjort att stamceller tilldragit sig stor uppmärksamhet under de senaste åren. Man ser ett stort kliniskt värde i att få ökad kunskap om hur stamceller fungerar. Ett möjligt användningsområde är att de vid celltrans-

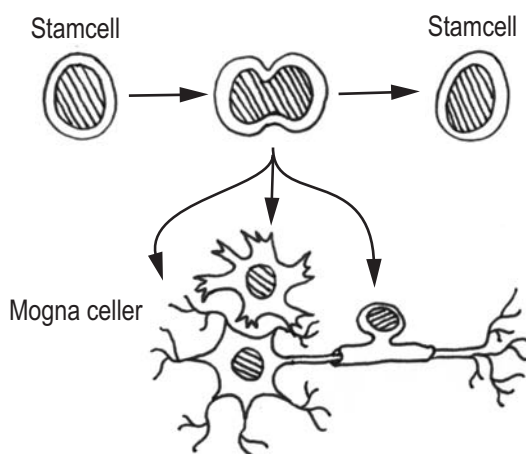


Figure 11. En stamcell kan göra en kopia av sig själv, samt bilda nya mogna celltyper.

mogen celltyp? Hur kan stamceller veta vilken sorts cell de ska bilda? De här frågorna är viktiga att besvara, både för att vi ska få ökad förståelse för hur CNS utvecklas och för att hitta bättre behandlingsmetoder mot sjukdomar och skador som drabbar CNS.

En stamcell påverkas av och kommunicerar med sin omgivning. Detta sker till stor del med hjälp av lösliga proteiner som frisätts från en cell och tas emot av en annan cell. Mottagandet av ett protein sker via s k receptorer, som sitter på ytan av cellen. Vi har studerat proteinet PDGF (platelet-derived

plantation skulle kunna ersätta skadade celler och därmed bota sjukdomar som diabetes och Parkinsons sjukdom.

Neuronala stamceller finns i det centrala nervsystemet (CNS - hjärnan och ryggmärgen) och kan utvecklas till nervceller och gliaceller. I det här avhandlingsarbetet har jag odlat neuronala stamceller från embryonal råtthjärna i laboratoriet (Figur 8) och sökt svaren på följande frågor: Vad är det som styr när en stamcell ska dela sig? Vad är det som styr när stamcellen ska bilda en

growth factor), som sedan tidigare är känt för att påverka bildningen av nervceller. Dessutom har ett överuttryck, d v s onormalt höga nivåer, av PDGF och PDGF-receptorer observerats i vissa hjärntumörer.

Neuronala stamceller som behandlats med PDGF bildar fler nervceller jämfört med obehandlade stamceller. I delarbete I visar vi att detta sker genom att PDGF instruerar omogna nervceller att dela sig. När PDGF binder till PDGF-receptorerna på stamcellens yta aktiveras signalproteiner som finns inne i cellen. Dessa bildar ett komplicerat signaleringsnätverk, vars ändamål är att aktivera gener i cellkärnan (Figur 7). Bara en aktiverad gen kan bilda ett nytt protein.

Ett intracellulärt signalprotein, som ansetts vara viktigt för nervcellsbildning, är ERK (extracellular signal-regulated kinase). Vi ville undersöka om ERK-signalering är nödvändig för neuronala stamcellers mognad till nervceller. Dessutom ville vi veta om PDGF aktiverar ERK. I delarbete II påvisas att ERK inte behövs när stamceller ska bilda mogna celltyper. Däremot verkar ERK vara viktig för PDGFs celldelningseffekt på omogna nervceller.

Vi undersökte i delarbete III vilka gener som slås på när neuronala stamceller behandlas med PDGF. Genom att avläsa genaktiviteten drar vi slutsatsen att PDGF-behandlade celler är ett mellanting mellan neuronala stamceller och mogna celler.

Vad händer om det finns för stor mängd av PDGF i det centrala nervsystemet under fosterutvecklingen? Kommer det att bildas fler nervceller än normalt? Kommer det att bildas hjärntumörer? För att försöka besvara dessa frågor användes, i delarbete IV, transgena möss (Figur 10). Ordet transgen betyder i det här fallet att mössen fått en extra PDGF-gen införd i sina neuronala stamceller, vilket leder till att de bildar mer PDGF-protein än normalt. Vi kunde inte se någon skillnad i antalet nervceller hos transgena musembryon jämfört med normala musembryon. Däremot kunde en ökad mängd döende celler observeras i de transgena mushjärnorna. En möjlig förklaring till detta kan vara att överuttrycket av PDGF har lett till att fler nervceller har bildats, men att hjärnans eget skyddssystem själv kan ta hand om detta cellöverflöd. I vår fortsatta studie ska vi undersöka om vuxna transgena mushjärnor är normala eller ej, med avseende på mängden celler som bildats. Vi kommer även att studera huruvida vuxna transgena möss lättare utvecklar hjärntumörer än icke-transgena möss.

För att förstå hur det centrala nervsystemet bildas är det viktigt att identifiera signaler som reglerar neuronala stamceller under fosterutvecklingen. Utökad kunskap inom detta område kan också ge oss inblick i hur vissa hjärntumörer hos barn bildas, då dessa antas uppkomma från stamceller. Intressant är att dessa tumörer ofta innehåller onormalt hög mängd PDGF.

Resultaten som presenteras i den här avhandlingen visar att PDGF har en betydande roll för reglering av stamceller i det centrala nervsystemet.

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