Stem Cells and Cancer Stem Cells 4
Therapeutic Applications in Disease and Injury

M.A. Hayat
Editor

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Although touched by technology, surgical pathology always has been, and remains, an art. Surgical pathologists, like all artists, depict in their artwork (surgical pathology reports) their interactions with nature: emotions, observations, and knowledge are all integrated. The resulting artwork is a poor record of complex phenomena.

Richard J. Reed, MD

One Point of View

All small tumors do not always keep growing, especially small breast tumors, testicular tumors, and prostate tumors. Some small tumors may even disappear without a treatment. Indeed, because prostate tumor grows slowly, it is not unusual that a patient may die at an advanced age of some other causes, but prostate tumor is discovered in an autopsy study. In some cases of prostate tumors, the patient should be offered the option of active surveillance followed by PSA test or biopsies. Similarly, every small kidney tumor may not change or may even regress. Another example of cancer or precancer reversal is cervical cancer. Precancerous cervical cells found with Pap test, may revert to normal cells. Tumor shrinkage, regression, reversal, or stabilization is not impossible.

Another known example of cancer regression is found in pediatric neuroblastoma patients. Neuroblastoma shows one of the highest rates of spontaneous regression among malignant tumors. In addition to the well-known spontaneous regression in stage 4S disease, the high incidence of neuroblastoma remnants found during autopsy of newborns suggest that localized lesions may undergo a similar regression (Guin et al. 1969). Later studies also indicate that spontaneous regression is regularly seen in infants with localized neuroblastoma and is not limited to the first year of life (Hero et al. 2008). These and other studies justify the "wait and see" strategy, avoiding chemotherapy and radiotherapy in infants with localized neuroblastoma, unless *MYCN* gene is amplified. Infants with nonamplified *MYCN* and hyperdiploidy can be effectively treated with less intensive therapy. Infants with disseminated disease without *MYCN* have excellent survival with minimal or no treatment.

The pertinent question is: Is it always necessary to practice tumor surgery, radiotherapy, or chemotherapy? Although the conventional belief is that cancer represents an "arrow that advances unidirectionally", it is becoming clear that for cancer to progress, they require cooperative microenvironment (niche), including immune system and hormone levels. However, it is emphasized that advanced (malignant) cancers do not show regression, and require therapy. In the light of the inadequacy of standard treatments of malignancy, clinical applications of the stem cell technology need to be expedited.

Eric Hayat

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Preface

This is volume 4 of the seven-volume series, "Stem Cells and Cancer Stem Cells: Therapeutic Applications in Disease and Tissue Injury." A stem cell is defined as a cell that can self-renew and differentiate into one or more specialized cell types. A stem cell may be pluripotent, which is able to give rise to the endodermal, ectodermal, and mesodermal lineages; an example is embryonic stem cells. A stem cell may be multipotent, which is able to give rise to all cells in a particular lineage; examples are hematopioietic stem cells and neural stem cells. A stem cell may be unipotent, which is able to give rise to only one cell type; an example is keratinocytes.

A cancer stem cell is a cell type within a tumor that possesses the capacity of self-renewal and can give rise to the heterogeneous lineages of cancer cells that comprise the tumor. In other words, a cancer stem cell is a tumor initiating cell. A unique feature of cancer stem cell is that although conventional chemotherapy will kill most cells in a tumor; cancer stem cells remain intact, resulting in the development of resistance of therapy. All of these types of stem cells are discussed in this volume.

Vast therapeutic applications of the following specific stem cells in disease and tissue injury are discussed: embryonic stem cells, induced pluripotent stem cells, human hair follical stem cells, bone marrow-derived human mesenchymal stem cells, adipose-derived stem cells, periodontal/perogenitor cells, cancer stem cells, and breast cancer stem cells.

As stated above, given that human embryonic stem cells possess the potential to produce unlimited quantities of any human cell type; considerable focus has been placed on their therapeutic potential. Because of the pluripotency of embryonic stem cells, they have been used in various applications such as tissue engineering, regenerative medicine, pharmacological and toxicological studies, and fundamental studies of cell differentiation. The formation of embryoid bodies, which are three-dimensional aggregates of embryonic stem cells, is the initial step in the differentiation of these cells. As stated above, embryonic stem cells can differentiate into derivatives of three germ layers: the endoderm, mesoderm, and ectoderm. Therefore, embryoid body culture has been widely used as a trigger for the *in vitro* differentiation of embryonic stem cells.

Role of cancer stem cells, specifically in breast cancer is explained. Transplantation of mesenchymal stem cells to aid the injured brain is included. Immune recovery after stem cell transplantation in severe combined immunodeficiency patients is described. Role of mesenchymal stem cells in enhancing

the growth and metastasis of colon cancer is discussed. Clinical application of human follical stem cells as marker is presented. Treatment of malignant gliomas using genetically-modified neural stem cells is discussed. The impact of cancer stem cells hypothesis on designing new cancer therapies is explained. In the field of regenerative medicine, the use of stem cells in the repair of the central nervous system, tendon injury, and as a cardiac regenerative medicine is described. Role of DNA methylation in maintaining stemness induced pluripotent stem cells from human extraembryonic amnion cells is discussed. Insights on the understanding of molecular pathways involved in tumor biology are explained, which lead to the development of effective drugs. Information on pathways (e.g., hedgehog) facilitates targeted therapies in cancer.

By bringing together a large number of experts (oncologists, neurosurgeons, physicians, research scientists, and pathologists) in various aspects of this medical field, it is my hope that substantial progress will be made against terrible human disease and injury. It is difficult for a single author to discuss effectively the complexity of diagnosis, therapy, including tissue regeneration. Another advantage of involving more than one author is to present different points of view on a specific controversial aspect of cancer cure and tissue regeneration. I hope these goals will be fulfilled in this and other volumes of the series. This volume was written by 71 contributors representing 11 countries. I am grateful to them for their promptness in accepting my suggestions. Their practical experience highlights their writings, which should build and further the endeavors of the readers in these important areas of disease and injury. I respect and appreciate the hard work and exceptional insight into the nature of cancer and other disease provided by these contributors. The contents of the volume are divided into four subheadings: Molecular Genetics, Regenerative Medicine, Therapy, and Transplantation for the convenience of the readers.

It is my hope that subsequent volumes of the series will join this volume in assisting in the more complete understanding of the causes, diagnosis, and cell-based treatment of major human diseases and debilitating tissue/organ injuries. There exists a tremendous, urgent demand by the public and the scientific community to address to cancer diagnosis, treatment, cure, and hopefully prevention. In the light of existing cancer calamity, government funding must give priority to eradicating deadly malignancies over military superiority.

I am thankful to Dr. Dawood Farahi and Dr. Kristie Reilly for recognizing the importance of medical research and publishing through an institution of higher education.

M.A. Hayat

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Part I

Molecular Genetic

Neural Stem/Progenitor Cell Proliferation and Differentiation: Role of Sonic Hedgehog and Wingless/Int-1 Proteins

1

Miroslava Anderova and Pavel Honsa

Abstract

The Sonic hedgehog and Wingless-Int protein (Wnt) signaling pathways have proven to be essential at various stages of neural development, but also in the ongoing neurogenesis of the adult hippocampus and subventricular zone under physiological conditions as well as in pathological states, such as traumatic brain injury, ischemia or neurodegenerative diseases. Here we review key findings demonstrating the role of Sonic hedgehog and Wingless-Int proteins (Wnts) in modulating the proliferation of neural stem/progenitor cells and affecting the fate decision of neural stem/progenitor cells during embryonic development. Moreover, we also review current findings elucidating the role of these morphogens during neonatal and adult neural stem cell differentiation and their possible role in adult neurogenesis induced by neurodegenerative disorders of the CNS.

Keywords

Morphogens • Sonic hedgehog • Wnts • Development • Mitogenic activity Progenitor survival • Fate decision • Adult neurogenesis • CNS disorders

Introduction

Neurons, astrocytes and oligodendrocytes are derived from a single precursor cell, termed a neural stem cell (NSC). NSCs are characterized by their ability to divide either symmetrically, giving rise to two identical stem cells, or asymmetrically, thus generating one identical stem cell and one more differentiated cell. Neural progenitor cells (NPCs) are also cells of the neural lineage; however, their division generates either more differentiated progenitors or postmitotic cells, namely neurons and glial cells. During the development of the central nervous system (CNS), the maintenance of a particular equilibrium between the proliferation of neural stem/progenitor cells (NS/PCs) and their differentiation into the enormous diversity of neurons, astrocytes and oligodendrocytes is

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fundamental for the appropriate regional patterning of the developing neural tube and for controlling the size of different CNS regions. In the adult CNS, NS/PCs have an important role, for example in learning and hippocampal plasticity, in addition to supplying neurons to the olfactory bulb that migrate from their niche in the subventricular zone (SVZ). In particular, the enhanced/decreased proliferation or altered differentiation potential of NS/PCs plays an important role in the progression of human CNS diseases, such as multiple sclerosis, Alzheimer's disease or Parkinson's disease, as well as in nervous tissue regeneration after ischemic or traumatic CNS injuries. Perturbed stem cell function, such as impaired self-renewal capacity due to cellular senescence, contributes to ageing and degenerative diseases, while impaired stem cell differentiation by oncogenic mutations contributes to cancer formation. Large numbers of growth factors and signal transduction cascades have been shown to participate in controlling NS/PC behavior in the rodent and human CNS. Among these, Sonic hedgehog (Shh) and Wingless-Int proteins (Wnts) are thought to control the proliferation and fate of NS/PCs and their progeny. Their binding to specific cell surface receptors activates intracellular signaling pathways, which results in the promotion/repression of gene expression.

Sonic Hedgehog Signaling Pathways

Sonic hedgehog, a member of the Hedgehog family of secreted signaling proteins, carries out diverse functions during vertebrate development and adulthood. Three Hedgehog genes have been described in mice and humans: Shh, which is expressed in specific cell groups in many organs such as the brain and lungs, Indian hedgehog, which is prominently expressed in the bone, and Desert hedgehog, which is found in the gonads. Shh-secreting cells express the transmembrane protein Dispatched (Disp), which has a sequence very similar to that of the Shh-binding protein Patched (Ptc), the main Shh receptor. Disp is required in Shh-secreting cells, in contrast to Ptc, which is active in recipient cells.

Patched, a 12-transmembrane domain receptor that binds Shh with nanomolar affinity, is required for the repression of target genes in the absence of Shh (Fig. 1.1). The Shh signal induces the target genes by binding and inactivating Ptc. The inactivation of Ptc function allows another Shh receptor, Smoothened (Smo), to become active, which leads to the transcription of downstream genes. In humans and also in mice, the loss of Ptc function causes medulloblastomas, tumors of the cerebellum and many developmental abnormalities, all of which result from the inappropriate expression of Shh target genes. In addition to repressing target gene transcription, Ptc regulates the movement of Shh in tissues through the binding of Shh to Ptc and the subsequent internalization of this complex. This process is crucial in establishing and maintaining proper morphogen gradients.

While Ptc is the main repressor of Shh target gene transcription, Smo is their main positive activator. This 7-transmembrane protein is a close relative of the Frizzled proteins that act as Wnt receptors and is required in both vertebrates and invertebrates for Shh signal transduction. After binding Shh to Ptc, Smo is translocated from the endosomes to the primary cilium and becomes active, thereby decreasing the activity of protein kinase A. This leads to the inhibition of Gli transcription factor processing into their transcriptional repressor forms and the concomitant accumulation of transcriptional activators. Suppressor of Fused (SuFu), a negative regulator of Shh signaling, interacts with the three vertebrate Gli proteins (Gli1, Gli2, Gli3) to retain them in the cytosol. These proteins contain five zinc-finger DNA-binding domains and differ by their N-terminal domains. Gli1 is probably an amplifier of the Shh response rather than a direct effector of the Shh transduction machinery. However, Gli1 is a target gene of the pathway and is classically used as a convenient readout for pathway activation. Gli2 functions mainly as a transcriptional activator, but can also display repressor activity in specific contexts. Conversely, Gli3 mainly functions as a transcriptional repressor. An important role in the Shh signaling pathway is played by the primary cilium, a microtubule-based cell surface protrusion present in most mammalian cells. Mutations in several components of the intra-flagellar transport

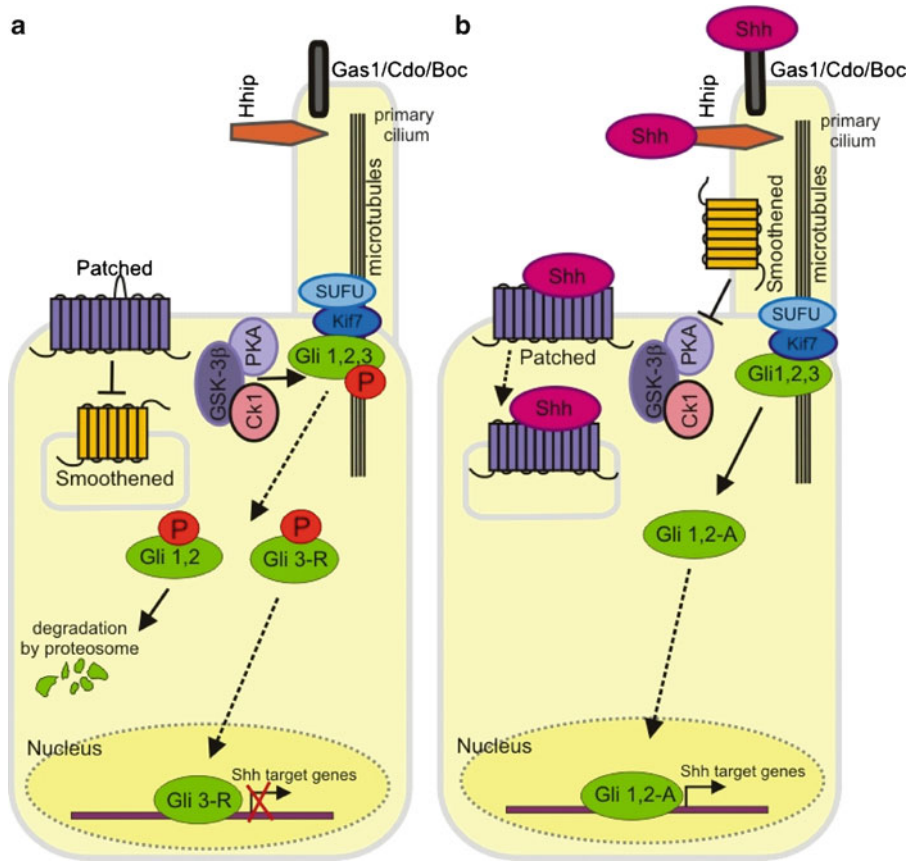

Fig. 1.1 Sonic hedgehog signaling in mammalian cells. Patched is normally bound to the cell membrane and prevents the membrane association of Smoothened, a G-coupled transmembrane protein. In the absence of Shh (**a**), Suppressor of Fused (*SuFu*) and Kinesin family member 7 (*Kif7*) sequester the microtubule-bound pool of the transcription factor Gli in the primary cilium. Gli can be phosphorylated (*P*) by protein kinase A (*PKA*), casein kinase-1 (*CK1*), and glycogen synthase kinase 3β (*GSK-3β*), resulting in the degradation of Gli activators (*Gli1* and *Gli2*) or the generation of repressor-Gli (*Gli3*), which in turn leads to the repression of Hedgehog target genes. In the presence of Shh (**b**), Patched after binding Shh enables the translocation of Smoothened to the primary cilium, where its associated G protein activity inhibits suppressive kinase action on Gli, leaving Gli free to translocate to the nucleus and activate Shh target genes. Shh also binds to Hedgehog interacting protein (*Hhip*), a molecule without receptor activity, which leads to the sequestration of Shh. Additionally, the receptors Gas1, Cdo and Boc are present on the cell surface, which enhances the response of the cell to low levels of Shh

machinery, which are required for the establishment and maintenance of cilia, affect Shh signaling in several developmental processes, including neural tube patterning, limb development, and adult neural stem cell formation (Traiffort et al. 2010). Moreover, all the major Shh pathway components, including Ptc, Smo, and Gli proteins, can be predominantly found in the cilia. In the absence of Shh, Ptc localizes to the cilia and prevents Smo from accumulating there; Shh binding to Ptc initiates the reciprocal transfer of Ptc and Smo, with Ptc moving out and Smo accumulating in the cilia. Smo constantly moves in and out of the cilia by binding to anterograde and retrograde molecular motors; Ptc and Shh signaling may control this balance by modulating Smo/molecular motor interactions. Shh probably induces the phosphorylation of Smo, which enhances its loading onto the anterograde motor and increases its concentration in the cilium. In addition to the foregoing canonical signaling pathway, several non-canonical mechanisms of

Shh signaling have also been described. These pathways do not comprise any Gli-mediated transcription and have been suggested for Shh-induced cell migration and axonal guidance. In the absence of Shh, Ptc may directly interact with Cyclin B1 or caspases, which inhibits cell proliferation or promotes apoptosis, respectively. Similarly, the repressor form Gli3 generated in the absence of Shh signaling is also able to inhibit canonical Wnt signaling (Ulloa and Briscoe 2007).

The Role of Sonic Hedgehog in Embryogenesis

Shh is expressed in the notochord, ventral to the neural tube, and in the ventral midline of the neural tube. Graded Shh signaling directs the patterning of the ventral neural tube and is critical for the generation of five ventral neuronal progenitor subtypes – three types of ventral interneurons, motor neurons, and glial-derived oligodendrocytes. The identity of these progenitors is imposed by specific combinations of transcription factors, which are regulated by different concentrations of Shh. The quantification of Shh distribution reveals that the gradient of Shh protein decays exponentially along the dorso-ventral axis. As expected, the most ventral progenitor cells are exposed to the highest levels of the ligand, while decreased ligand levels are observed across the motoneuron progenitor domain and the levels drop below detectability in the dorsal parts of the neural tube. Furthermore, cells close to the ventral midline are exposed to higher concentrations of Shh and for a longer period of time than more dorsal cells.

A question arose as to which mechanism is responsible for integrating the temporal variations in Shh concentration that lead to the generation of distinct quantities and durations of Gli activity. In vitro experiments indicate that short exposure to either high or low concentrations of Shh generates similar levels of Gli activity and leads to the expression of the same sets of genes, while long exposure to a constant amount of Shh decreases the levels of Gli activity in cells. In other words, relatively low concentrations of Shh are sufficient to activate the highest levels of signal transduction; however, with increasing exposure time, cells become desensitized to constant Shh signaling. In this way, cells convert a concentration of Shh into a proportional period of Gli activity. The progressive increase in the length of these periods in response to a higher Shh concentration correlates with the sequential appearance of more ventrally expressed gene profiles. The most important feature of this mechanism is that changing the concentration and/or the duration of Shh exposure has a similar effect on gene expression and subsequent differentiation.

Two sets of proteins are responsible for this mechanism. One set, consisting of the proteins Gas1, Cdo and Boc, is expressed on the cell membrane in the absence of Shh ligand and enhances the cellular response to low levels of Shh. The expression of these proteins is markedly reduced shortly after activation of the Shh signaling pathway, decreasing the sensitivity of the cells to Shh. The other set of proteins comprises Ptc and Shh-binding protein without receptor activity – Hhip1. Their levels are increased after activation of the Shh signaling pathway, leading to the sequestration of Shh in the case of Hhip1 and to the inhibition of Smo in the case of Ptc. The sensitization/desensitization of the cellular responses to Shh levels is also supported by the direct activation of the Smo receptor via its specific activator. This Smo receptor activation is independent of Shh levels and induces a different profile of Gli activity compared to that generated by pathway activation with Shh protein. This complex regulatory system might act as a buffering system to increase signaling when levels of Shh are low and reduce signaling in cells exposed to high ligand levels. This may compensate for fluctuations in ligand availability, particularly at early stages of neural tube development, thereby rendering some robustness to gradient interpretation (Ribes and Briscoe 2009).

In addition to its instructive role in embryonic stem cell differentiation, Shh signaling is an important mitogenic factor during embryogenesis. Shh activity promotes the proliferation of embryonic neural stem/progenitor cells in the cerebellum, tectum, neocortex and spinal cord. In both the chick and mouse spinal cord, activation of the Shh sig-

naling pathway increases progenitor proliferation and consequently the growth of the neural tube. There exist many connections between the Shh signaling pathway and regulators of the G1/S transition (cyclin D1, N-Myc and Bcl-2 are direct targets of Shh signaling). Moreover, Shh signaling upregulates the G2/M regulator CDC25B110 and the polycomb group protein Bmi1 (for the review see Traiffort et al. 2010).

The development of the neocortex is another well-described and illustrative example of the role of Shh during mammalian embryogenesis. Radial glial cells play a pivotal role in the formation of the six-layered structure of the mammalian neocortex. They serve as a scaffold for newly derived neurons that attach to their processes and migrate to the most superficial lamina. These processes spread between the inner ventricular zone and the surface of the developing cortex. At the same time, radial glial cells divide and give rise to new neuronal progenitor cells and to radial glial cells. In the case of generating neuronal progenitor cells, asymmetric division takes place, while symmetric division is responsible for the production of new radial glial cells. This process is strictly controlled, and the Shh signaling pathway plays a major role together with Notch signaling. At embryonic days 9.5–14.5, Shh supports the symmetric proliferative division of radial glial cells. This increases the pool of radial glial cells, but in the case of inappropriate activation, it does not lead to proper neuronal development. A counterbalance to Shh signaling is the Notch pathway, which conversely enhances asymmetric proliferative division and maintains the balance between the symmetric and asymmetric division of radial glial (Dave et al. 2011).

Moreover, Shh promotes the survival of neural progenitor cells during embryogenesis. The removal of the notochord and floor plate of the neural tube results in a massive apoptotic response in progenitors. This phenomenon can be reversed by delivering an exogenous source of Shh. Embryonic neural stem cells have an activated ready-to-run death program, which must be actively inhibited by Shh signaling. This inhibition enables the survival and subsequent differentiation of embryonic neural stem cells (Charrier et al. 2001). In the neural tube, both the mitogenic and survival activity of Shh are cell-autonomous and can be accounted for by the action of Gli proteins. Thus, the expression of Gli1, or a dominant active Gli protein that acts as a constitutive transcriptional activator, increases the proliferation of neural progenitors in the chick neural tube. Conversely, the overexpression of a repressor form of Gli3, which inhibits the activation of Shh responsive genes, decreases cell proliferation and promotes cell death. Together, the data suggest a model in which the absence of Shh signaling raises the levels of repressor forms of Gli proteins – mainly the repressor forms of Gli3 – to a point that inhibits the expression of genes necessary to promote cell cycle progression and cell survival (Ulloa and Briscoe 2007).

Sonic Hedgehog in Neonatal Neural Stem/Progenitor Cells

The Sonic hedgehog signaling pathway also assumes control of many processes in neonatal neural stem/progenitor cells shortly after birth. In NS/PCs that were isolated from the mouse neonatal forebrain and transduced with a plasmid carrying the Shh gene, Shh significantly promotes the proliferation rate in vitro. The NS/PCs that are kept in proliferative conditions generate large and fast growing neurospheres. Under differentiation conditions, Shh increases the survival rate of NS/PCs and preserves their undifferentiated phenotype by blocking neuronal differentiation (Prajerova et al. 2010). Additionally, Shh plays an important role in controlling the development of the cerebellum. This process is initiated in the embryonic mammalian brain, but its major growth phase occurs in late gestation and continues through the neonatal period. Granule neurons are the most numerous cells in the cerebellum. During development, they comprise the external granule layer of the cerebellum and proliferate extensively under the control of Shh molecules that are produced by Purkinje cells. In rodents, cerebellar granular stem progenitor cells undergo their major expansion from P0 to P14, while in humans, cerebellar growth continues until at least 1 year of age (Kenney et al. 2004).

The Role of Sonic Hedgehog in the Adult Brain

The discovery of newly derived neuronal cells in an adult mammalian brain initiated a search for substances that control the process of adult neurogenesis. One of the first candidates were morphogens that control the behavior of NS/PCs during the embryonic phase of life. Many studies have confirmed the expression of morphogen signaling pathway components in adult neurogenic regions – the subventricular zone (SVZ) of the lateral ventricles (LV) and the subgranular zone (SGZ) of the gyrus dentatus in the hippocampus. To date, a large number of substances that influence the process of adult neurogenesis have been determined; however, morphogens still play one of the most important roles in this process.

Sonic Hedgehog Signaling in the Subventricular Zone

The SVZ of the LV is one of the main neurogenic regions generating new neurons in the adult mammalian brain. Quiescent astroglial-like stem cells (type B cells) give rise to transient-amplifying cells (type C cells), which are the precursors of neuroblasts (type A cells). The neuroblasts migrate throughout the rostral migratory stream (RMS) to the olfactory bulbs (OB), where they differentiate into interneurons and integrate into the existing neuronal network. In the postnatal rodent SVZ, the main Shh signaling pathway components have been detected in dissociated and sorted cells with a cell-type specific distribution (Palma et al. 2005). Shh has been found in the rodent SVZ and in the cerebrospinal fluid (CSF) by Western blot analysis. Despite the fact that RT- PCR analysis has demonstrated that Shh is expressed in the SVZ, RMS, and OB, the phenotype of the Shh-producing cells has not yet been identified. This is probably a result of the weak signals obtained using in situ hybridization in the SVZ, which precludes co-localization experiments with specific markers (Palma et al. 2005). However, there is a possibility that Shh is not produced in the SVZ, but is imported from distant areas by, for example, the CSF. One of the suspected regions for Shh production is the wall of the third ventricle, where many cells display high Shh expression (Traiffort et al. 2010).

The expression of Gli1 transcription factor was found in quiescent neural stem cells in the SVZ and transient-amplifying cells using Gli1-CreERT2: R26R mice. Cells in this mouse strain express inducible Cre recombinase under the control of the Gli1 promotor, which enables the visualization and tracking of Shh-responding cells after breeding with the reporter mouse strain. From these cells arise many neuroblasts that migrate mainly into the OB (Ahn and Joyner 2005). Similar results were obtained with RT-PCR analysis of sorted type B and type C cells, which express high levels of Gli1, Gli2 and Ptc (Palma et al. 2005). In addition, type A cells express Ptc and Smo transcripts in the SVZ, RMS and OB, based on in situ hybridization (Traiffort et al. 2010). Gain- and loss-of-function experiments using the adenoviral transfer of Shh or transgenic mice with conditionally removed Shh signaling pathway components have suggested that Shh, identified in the SVZ and CSF, plays an important role in controlling the proliferation, differentiation, survival and migration of adult NS/PCs.

In experiments using the transgenic mouse strain $Nestin^{Cre/+};Smo^{c/c}$, an important component of the Shh signaling pathway – the Smo receptor – is removed, specifically in nestin-positive cells, which can be detected from embryonic day E12.5. The SVZ develops normally; however, a depletion of the quiescent B stem cell population and transient-amplifying C cells was found after birth. In contrast, the type A cell population expands precociously, but mostly fails to migrate to the OB. Finally, this neuroblast population is also depleted shortly after birth. These results imply that Shh signaling does not play a crucial role in prenatal SVZ establishment, but may have important functions in supporting postnatal neurogenesis in at least two different ways. First, Shh may function as a mitogen to promote the active proliferation of transient-amplifying C cells. Second, it may act as an important factor for the

maintenance of the self-renewing, slowly dividing B cell population. Additionally, Shh plays a role in proper neuroblast migration from the place of origin to the site of final differentiation (Balordi and Fishell 2007b).

In a similar study, the Smo receptor was conditionally removed from the stem cell niches in adult animals by using the tamoxifen-inducible transgenic Nestin$^{CreERT2/+}$;Smo$^{c/c}$ mouse strain. In those mice, the Smo receptor was removed by the injection of tamoxifen 60 days after birth. Both proliferation and neurogenesis were changed and failed to recover within 10 months following the conditional inactivation, indicating that Shh signaling is necessary for the maintenance of the quiescent B stem cell population and the proliferation of transient-amplifying cells during adulthood, not only for the establishment of the adult stem cell niche shortly after birth (Balordi and Fishell 2007a).

Furthermore, Shh possesses chemo-attractive activity, which is responsible for the control of migrating neuroblasts exiting the SVZ and then reaching the OB. The expression of Ptc and Smo was detected on PSA-NCAM-positive neuroblasts in the RMS. Blocking Shh activity in vivo by its physiological antagonist Hhip1 or neutralizing antibodies induces a significant decrease in the number of proliferating cells in the SVZ and, in parallel, an increase in the OB without affecting the survival or phenotype of the cells reaching the OB. Conversely, the adenoviral transfer of Shh and overexpressing Shh in the SVZ leads to an increase in the number of proliferating cells in the SVZ and a decrease in the OB. Furthermore, Shh displays chemo-attractive activity on neuroblasts in vivo, since Shh-expressing cells grafted above the RMS of adult mice induce migrating neuroblasts to deviate from their normal route (Traiffort et al. 2010).

Sonic Hedgehog Signaling in the Hippocampus

Neurogenesis in the hippocampus, the other most important region of adult neurogenesis, is very similar to that in the SVZ; however, the migration distance is significantly shorter and ends in the granular cell layer, where neuroblasts differentiate into mature granule neurons. Similarly to the SVZ, Shh and its signaling pathway components have been identified in the neurogenic regions of the hippocampus.

The source of Shh in the SGZ is probably Sox2-positive adult neural stem cells. In mutants with knocked-out Sox2 transcription factor, adult hippocampal neurogenesis is completely lost, leading to dentate gyrus hypoplasia. This process can be partially reversed by adding Shh pharmacological agonists. Moreover, chromatin immunoprecipitation identified the Shh gene promotor as a Sox2 target, and Sox2-deleted neural stem cells do not express Shh in vitro and rapidly die (Favaro et al. 2009).

The expression of Gli1 was found in quiescent adult neural stem cells type B, transient-amplifying cells type C as well as in newly derived neuroblasts (Ahn and Joyner 2005). Moreover, the Shh receptor Smo is expressed in type B cells in the gyrus dentatus and conditional ablation of this receptor in hGFAP::^{Cre}; Smo$^{fl/fl}$ mice leads to a deficit in the expansion of granule neuron precursors, and the dentate gyrus is severely hypotrophic. The same set of experiments revealed a crucial role of the primary cilia in controlling hippocampal neurogenesis through the Shh signaling pathway. After conditional removal of a subunit of the kinesin-II motor that is essential for ciliogenesis (Kif3a) in hGFAP::^{Cre}; Kif3a$^{fl/fl}$ mice, impaired Shh signaling in neural stem cells leads to the defective proliferation of type B cells and to inhibited differentiation into neuroblasts. Proliferation in the SVZ is also reduced in hGFAP::^{Cre}; Kif3a$^{fl/fl}$ mice, but this reduction is not as large as that observed in the SGZ of the hippocampus (Traiffort et al. 2010). The importance of the primary cilia in Shh signaling has been further documented in experiments, in which even the constitutively active Smo receptor requires developed primary cilia for its proper function. Additionally, Shh signaling significantly regulates the survival rate of newly generated neuronal cells. In the adult SGZ, neural progenitor cells are continuously generated, but many newly born cells die shortly after birth, and

only some of them survive and differentiate into granular neurons. After the administration of chlorobenzothiophene-containing molecule, a Smo agonist that has been shown to activate the Shh signaling pathway, significantly more BrdU-positive neurons survived and were detected in the gyrus dentatus (Bragina et al. 2010).

The Role of Sonic Hedgehog After Neural Injury

The healthy adult mammalian brain reacts to neural injuries with changes in protein expression, cell death/proliferation, the activation of microglial cells, reactive gliosis and the remodeling of existing undamaged circuits. Additionally, brain injury leads, in the majority of cases, to increased SVZ and/or SGZ neural stem cell proliferation, and many recent studies have described the Shh signaling pathway as an important regulator of these processes. For example, electroconvulsive seizure (ECS) in adult rats induces grand mal seizures with tonic and clonic convulsive components, which subsequently lead to increased adult hippocampal neurogenesis. Simultaneously, the increased expression of Ptc receptor mRNA and the decreased expression of Smo receptor mRNA have been described. Blocking the Shh signaling pathway with cyclopamine leads to decreased hippocampal neurogenesis after injury, which confirms the role of Shh in this process. Nevertheless, attempts to find a connection between injury and the activation of the Shh pathway have failed. One candidate was the excitotoxic neurotransmitter glutamate. Although its levels are dramatically increased after ECS, blocking of the glutamate receptors does not change the rate of Ptc or Smo mRNA transcription (Banerjee et al. 2005).

Another study described the role of the Shh signaling pathway after transient hippocampal ischemia. Immediately after ischemia, an increase in the mRNA levels of Shh and Gli1 was found. Subsequently, Western blotting revealed a higher concentration of Shh protein 7 days after injury, while immunohistochemistry localized this increase predominantly to mature hippocampal neurons. Additionally, the infusion of Shh or cyclopamine into the lateral ventricle starting 3 days after ischemia resulted in the decreased or increased proliferation of neural precursor cells, respectively (Sims et al. 2009).

The Wnt Signaling Pathways

Wingless-Int proteins (Wnts) belong to a large family of secreted, cysteine-rich glycosylated proteins that play an important role in both the developing and mature nervous systems. They comprise a large family of protein ligands encoded by about 19 genes in vertebrates. Three different Wnt signaling pathways have been identified: the canonical Wnt/β-catenin pathway, the non-canonical planar cell polarity pathway and the Wnt/Ca^{2+} pathway; however, the majority of Wnt proteins activate gene transcription through the canonical signaling pathway, in which β-catenin, a multifunctional protein, is the key component (for review see Inestrosa and Arenas 2010). In the canonical Wnt/β-catenin pathway (Fig. 1.2), Wnt signaling is transduced into the cytoplasm by the interaction of Wnt proteins with the receptor protein Frizzled (Fz) and the low-density lipoprotein receptor-related protein 5/6 (LRP5/6). This interaction activates Dishevelled (Dvl) by phosporylation, inactivates glycogen-synthase-kinase 3β (GSK-3β), a key modulator of the Wnt/β-catenin signaling pathway, and triggers the recruitment of Axin to the plasma membrane, resulting in the inhibition of β-catenin phosphorylation and degradation. Consequently, β-catenin accumulates in the cytoplasm and then translocates into the nucleus, where it forms a complex with the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, leading to the activation of the target genes. The Wnt pathway is tightly regulated at the receptor level by various regulatory proteins, such as LRP5/6 and Dickkopf (Dkk), and can be inhibited at different cellular levels. Members of a large family of secreted Frizzled-Related Proteins (sFRPs) bind directly to Wnt ligands, preventing them from interacting with Fz receptors. Interestingly, sFRPs contain a cysteine-rich